EPA
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ToxCast / Tox21 Data Overview

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National Center for Computational Toxicology

A Problem Driving CompTox Development

- ~1,000 chemicals – most people are often exposed to
- ~10,000 chemicals – many people are exposed to at least occasionally
- ~100,000 chemicals – some people are exposed to at least occasionally

- Some of these chemicals / exposures are responsible for
  - Cancers
  - Birth defects
  - Reproductive disorders
  - Both human and ecological species

- Can we prioritize chemicals for further testing without knowing everything?

ToxCast Projects

- ToxCast / Tox21 is a large-scale *in vitro* screening program
  - ToxCast: 1,000 chemicals in ~500 assays
  - Tox21: 10,000 chemicals in ~50 assays (drugs+EPA+NTP chemicals)

- Reverse toxicokinetics (RTK) approach for estimating maximum “no bioactivity” dose estimate

- Input for Endocrine Screening Prioritization and other programs

- Models for Prioritization of Targeted Testing
  - Test examples have known MOA, activity
  - Goal is to qualify *in vitro* assays for use in prioritizing testing of untested compounds

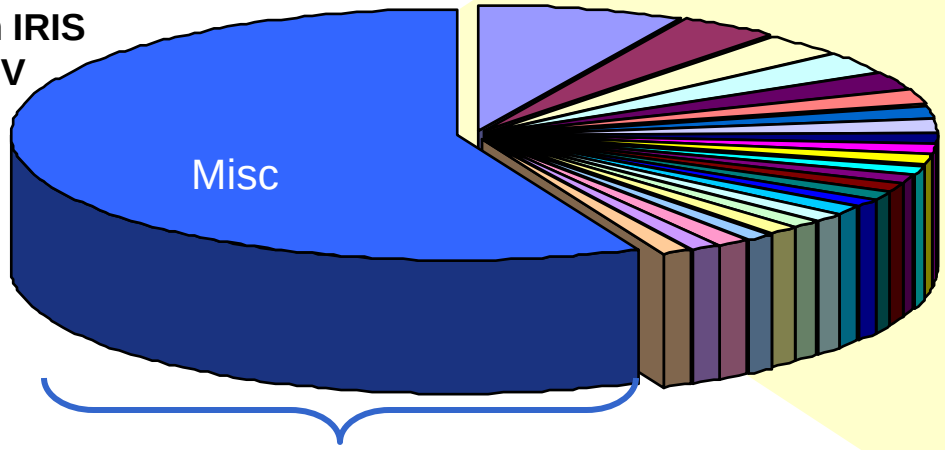
The ToxCast Phase I Chemicals (320)

309 Unique Structures
Replicates for QC

291 Pesticide Actives
9 Industrial Chemicals
8 Metabolites

56/73 Proposed Tier 1 EDSP

122 in IRIS
14 HPV



Misc MOA classes with
3 or fewer representatives

Classes with
> 3 chemicals

- **Acetylcholine esterase inhibitors**
- **conazole fungicides**
- **Sodium channel modulators**
- **pyrethroid ester insecticides**
- **organothiophosphate acaricides**
- **dinitroaniline herbicides**
- **pyridine herbicides**
- **thiocarbamate herbicides**
- **imidazolinone herbicides**
- **organophosphate insecticides**
- **phenyl organothiophosphate insecticides**
- **aliphatic organothiophosphate insecticides**
- **amide herbicides**
- **aromatic fungicides**
- **chloroacetanilide herbicides**
- **chlorotriazine herbicides**
- **growth inhibitors**
- **organophosphate acaricides**
- **oxime carbamate insecticides**
- **phenylurea herbicides**
- **pyrethroid ester acaricides**
- **strobilurin fungicides**
- **unclassified acaricides**
- **unclassified herbicides**

Classification based on OPPIN

ToxCast Assays

~500 Total Endpoints

Biochemical Assays

- Protein families
  - GPCR
  - NR
  - Kinase
  - Phosphatase
  - Protease
  - Other enzyme
  - Ion channel
  - Transporter
- Assay formats
  - Radioligand binding
  - Enzyme activity
  - Co-activator recruitment

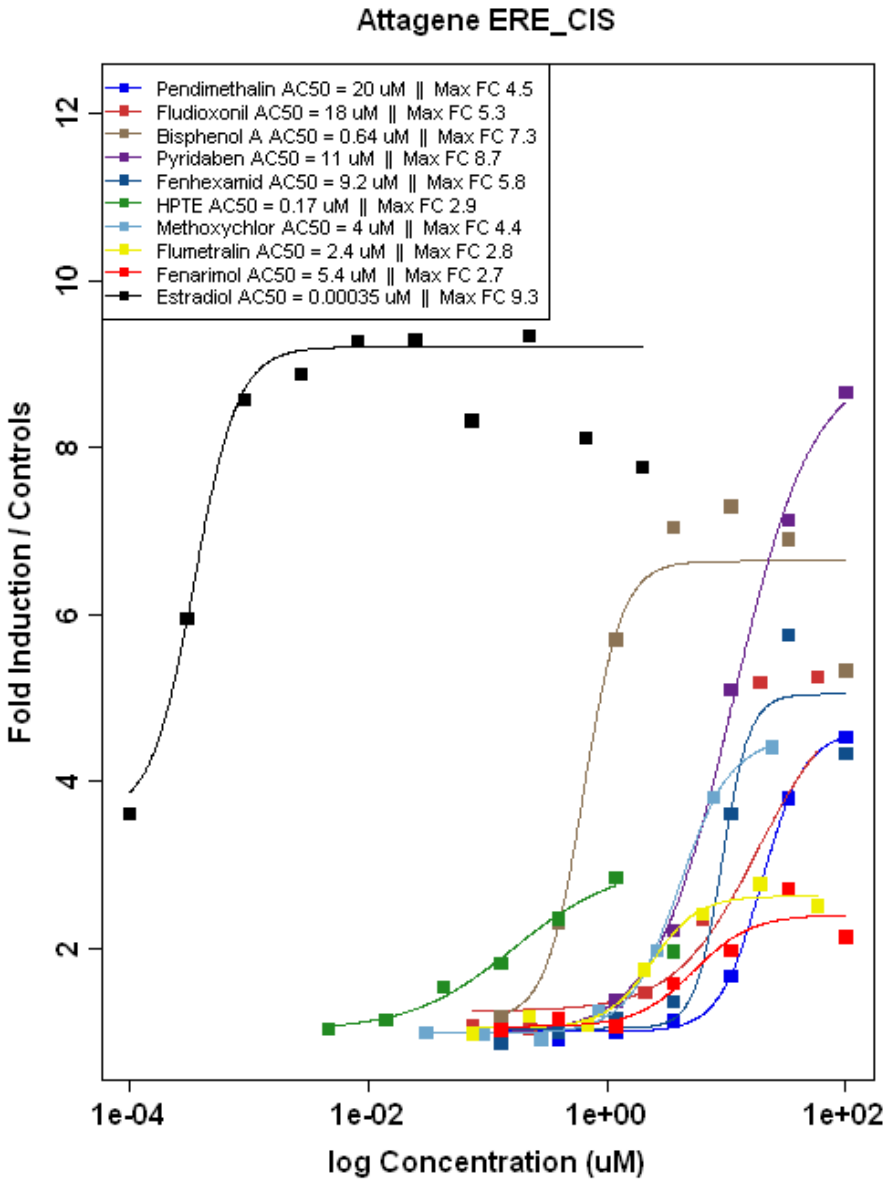
Primarily Human / Rat
Exception: Zebrafish development (Stephanie Padilla)

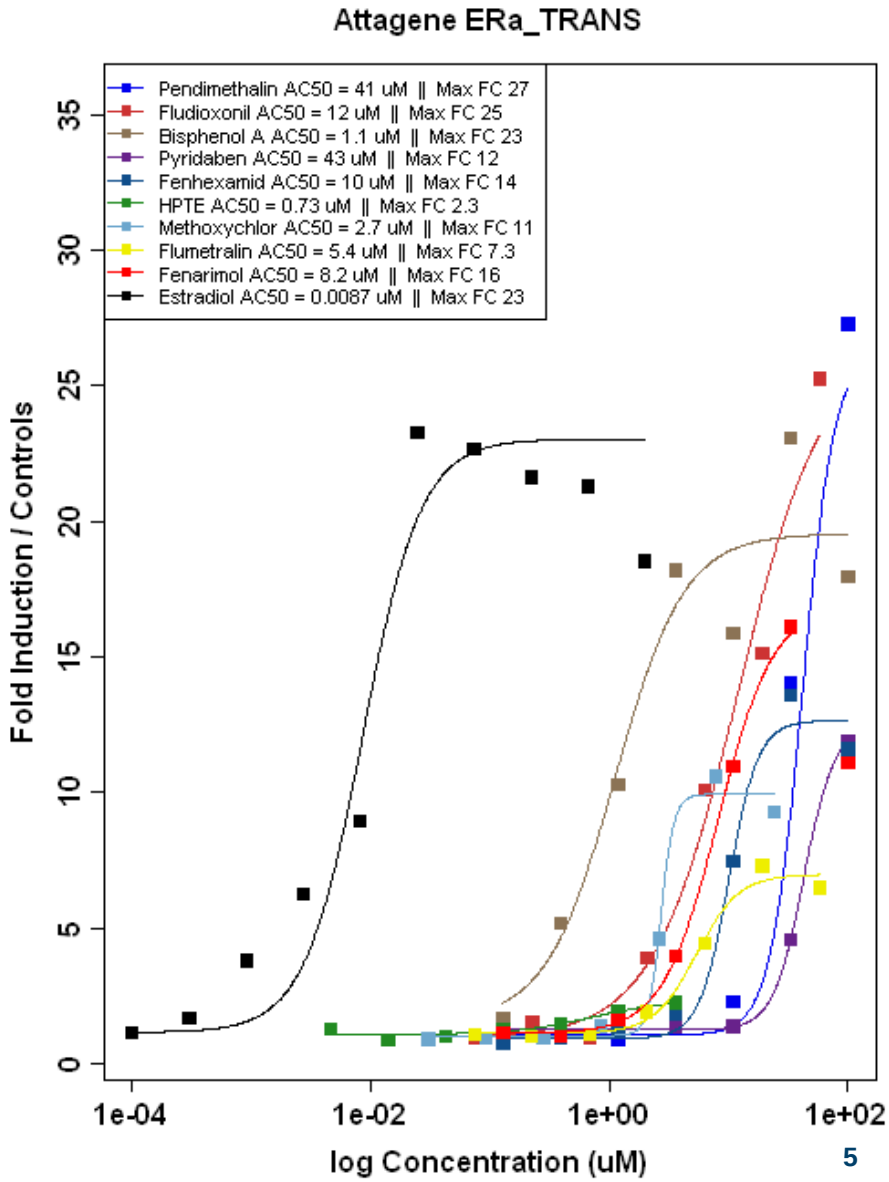
Cellular Assays

- Cell lines
  - HepG2 human hepatoblastoma
  - A549 human lung carcinoma
  - HEK 293 human embryonic kidney
- Primary cells
  - Human endothelial cells
  - Human monocytes
  - Human keratinocytes
  - Human fibroblasts
  - Human proximal tubule kidney cells
  - Human small airway epithelial cells
  - Rat hepatocytes
  - Mouse embryonic stem cells (Sid Hunter)
- Biotransformation competent cells
  - Primary rat hepatocytes
  - Primary human hepatocytes
- Assay formats
  - Cytotoxicity
  - Reporter gene
  - Gene expression
  - Biomarker production
  - High-content imaging for cellular phenotype

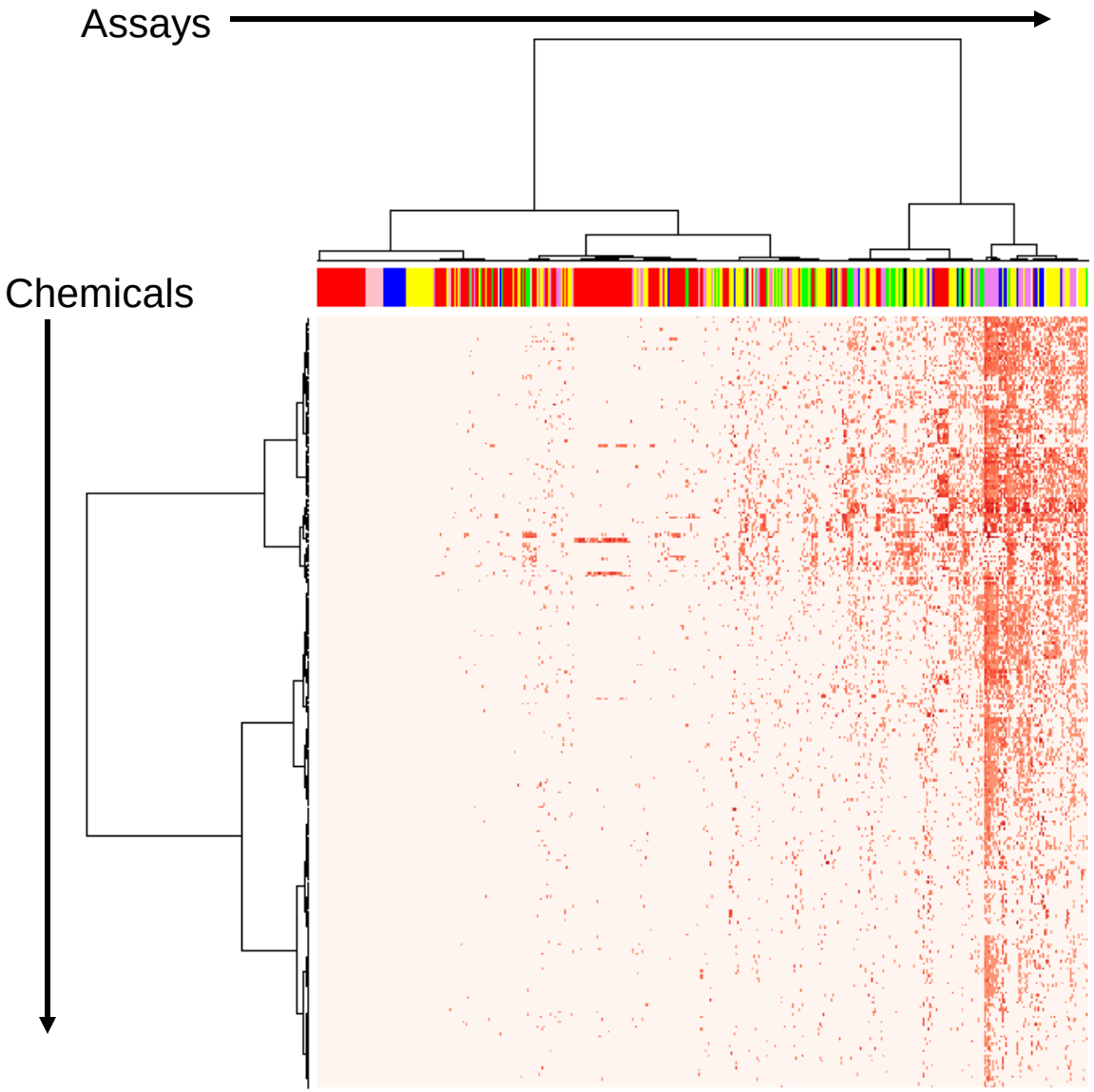
Data Analysis:

What is a hit?





The ToxCast In Vitro Data Set



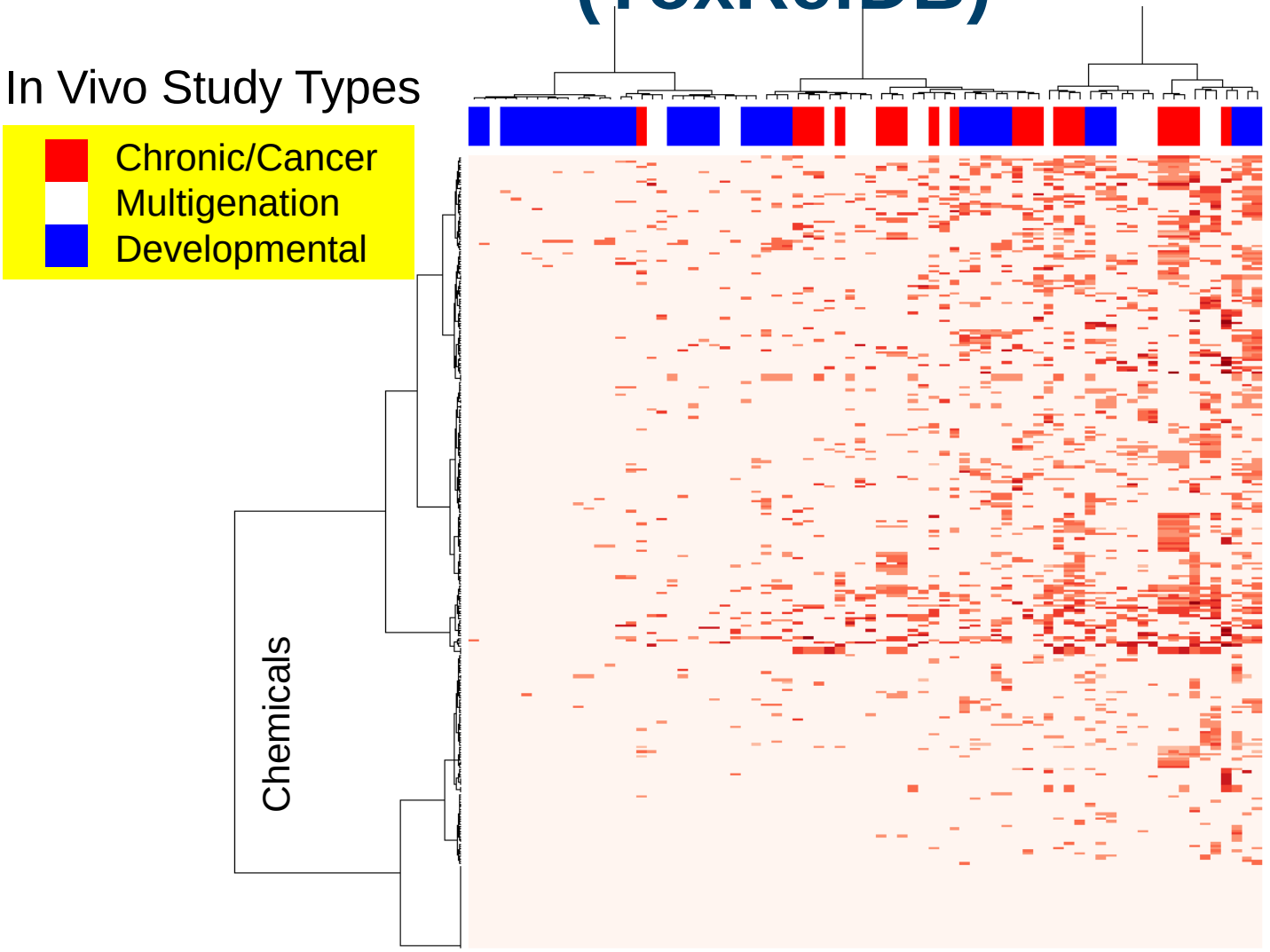
Many hits – median value=50

Fewer in cell-free HTS (Novascreen, red)

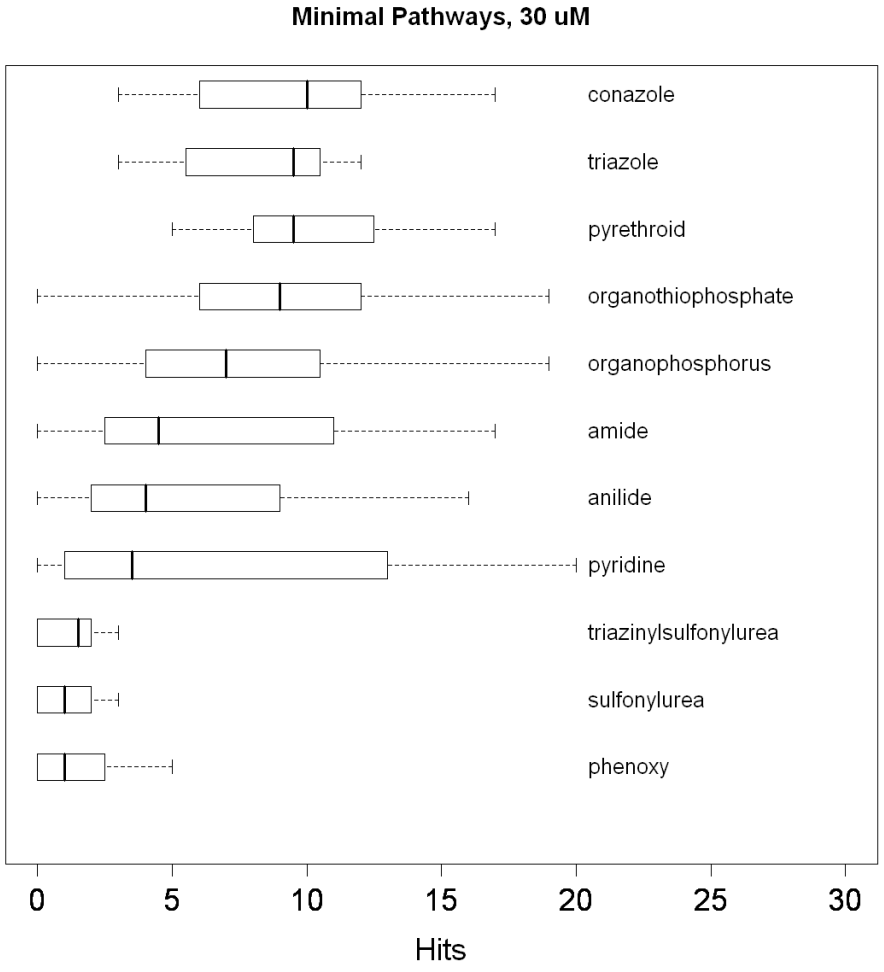
Many hits are at or near top of tested concentration range

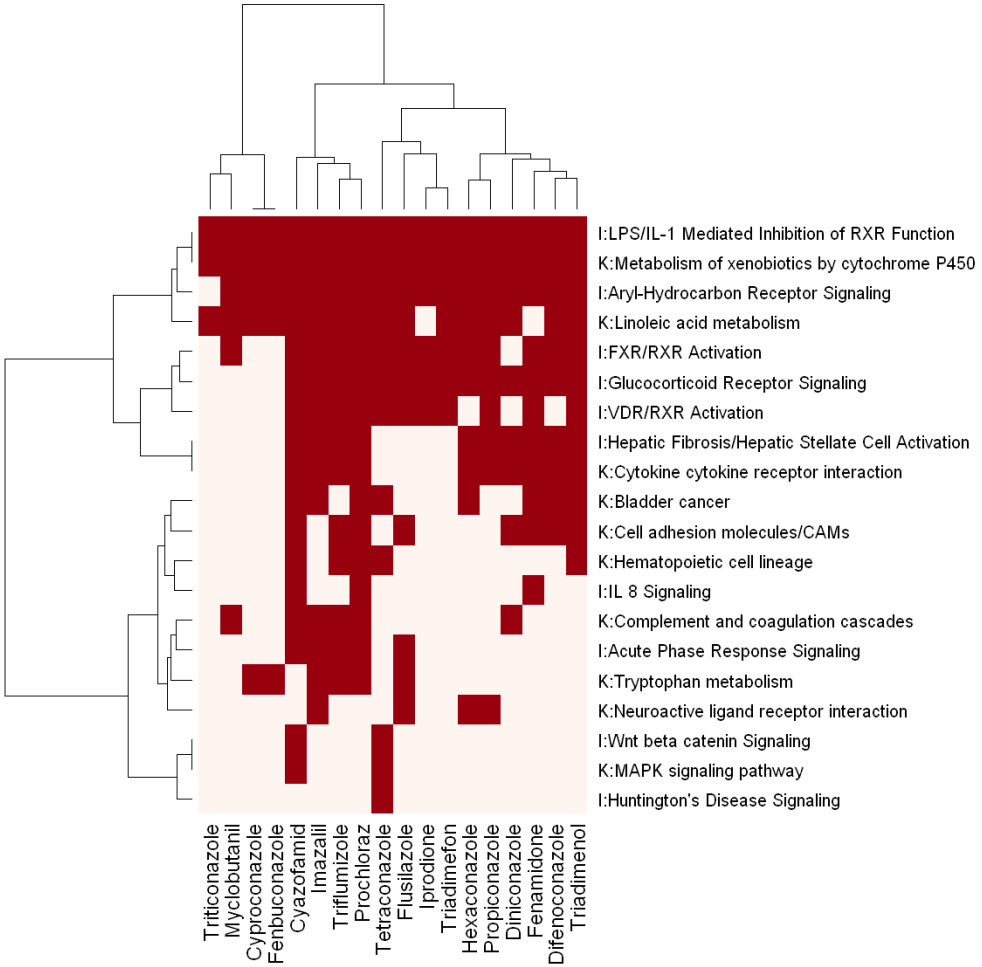
A few are in nanomolar range

Reference Toxicity Database (ToxRefDB)



Chemicals show a wide range of activity





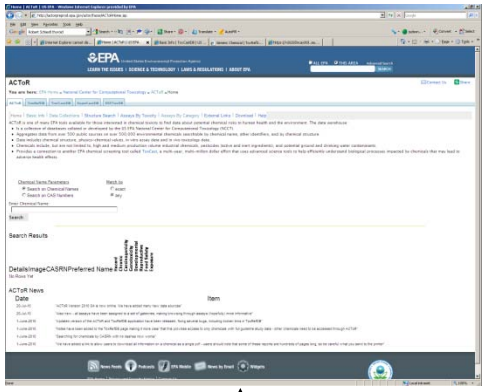
Where is the data?

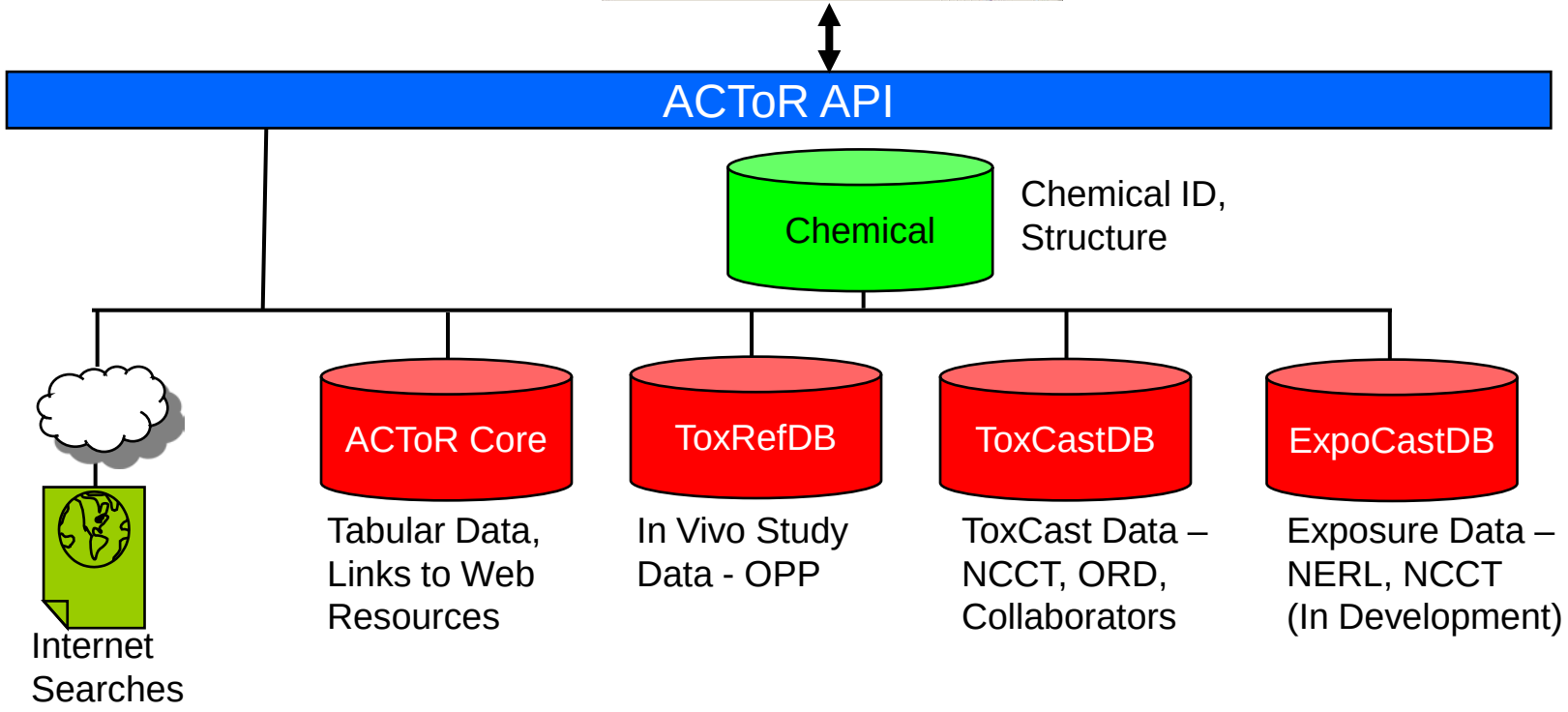
- ToxCastDB contains the ToxCast and ToxRefDB data for browsing
  - http://actor.epa.gov/actor/faces/ToxMiner/Home.jsp

- ACToR contains a much large amount of data on other types of toxicity and exposure
  - http://actor.epa.gov

ACToR
Aggregated Computational Toxicology Resource

http://actor.epa.gov/





Data Definitions

- Substance
  - A chemical from one source
  - Name(s), CASRN
  - Source-specific unique ID
  - Assay Data
- Compound
  - Chemical structure from one source
  - Source-specific unique ID
- Generic Chemical
  - CASRN
  - Link to many substance (each with same CASRN)
  - Link to at most one compound
  - Links to all assay data from substances with same CASRN

Data Definitions

- Assay
  - A collection of data on one or more substances
  - Comes from one data source
  - Can have several types of data included
  - Looks like an Excel spreadsheet
- Assay Component
  - One column of an assay table
- Assay Result
  - A data value for one substance and one assay component

Data Definitions

- Assay Phenotype
  - Type of disease associated with the assay
    - Carcinogenicity, GeneTox, …
- Assay Category
  - Type of data: tabular, links to the web, human exposure
  - Allows assays to be grouped together
- Data Collection
  - A source of data
  - Substances
  - Compounds
  - Assays

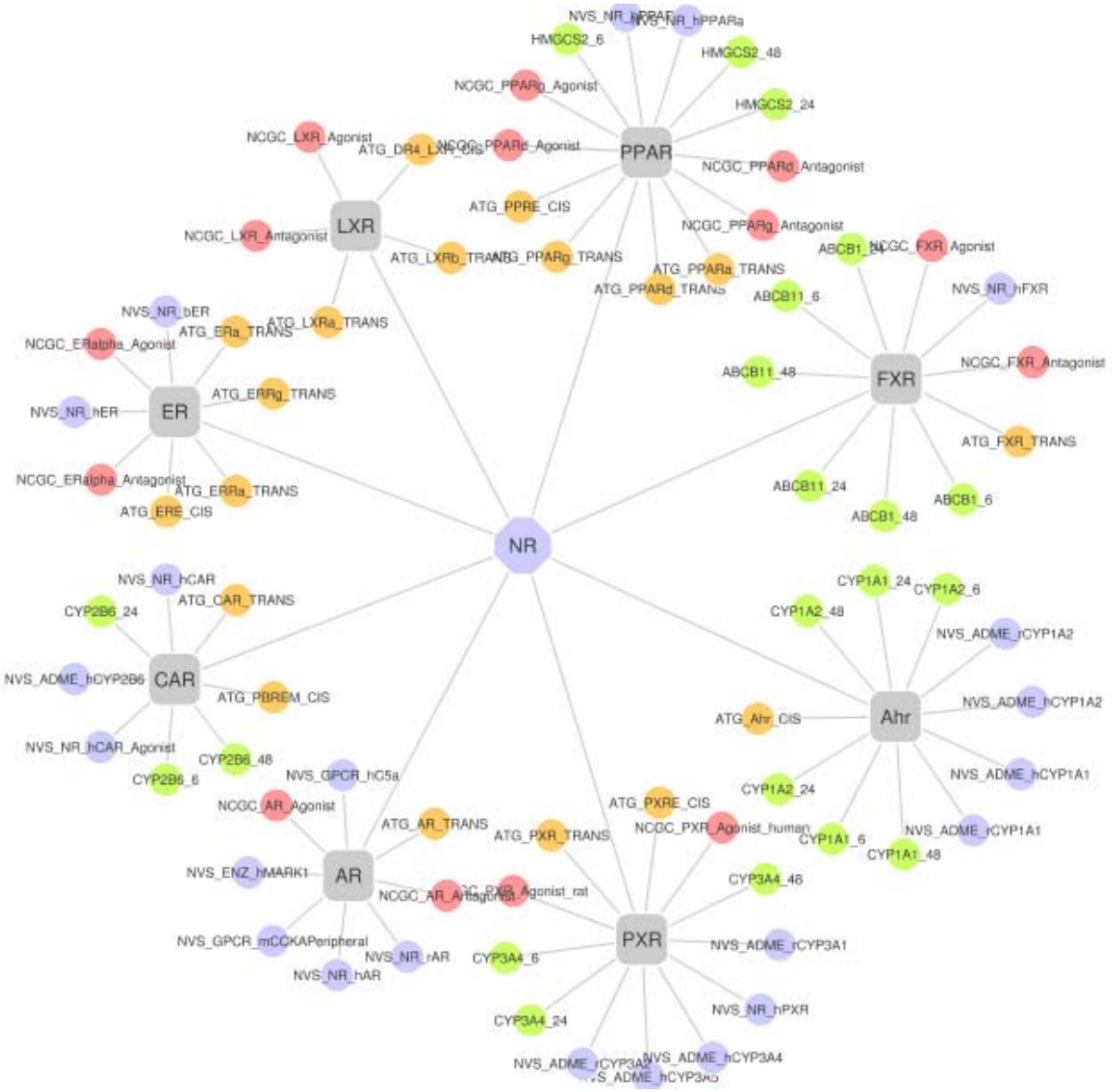
Data Sets

1. Attagene
2. ACEA
3. BioSeek
4. Cellumen
5. Gentronix
6. NCGC
7. Novascreen
8. CellzDirect
9. Solidus
10. ToxRefDB

- Perturbation Scores
  - Genes
  - Pathways
    - Ingenuity
    - KEGG
    - PathwayCommons
- PhysChem Properties
  - EPISuite
  - LeadScope
  - QikProp
- Structure Classifiers

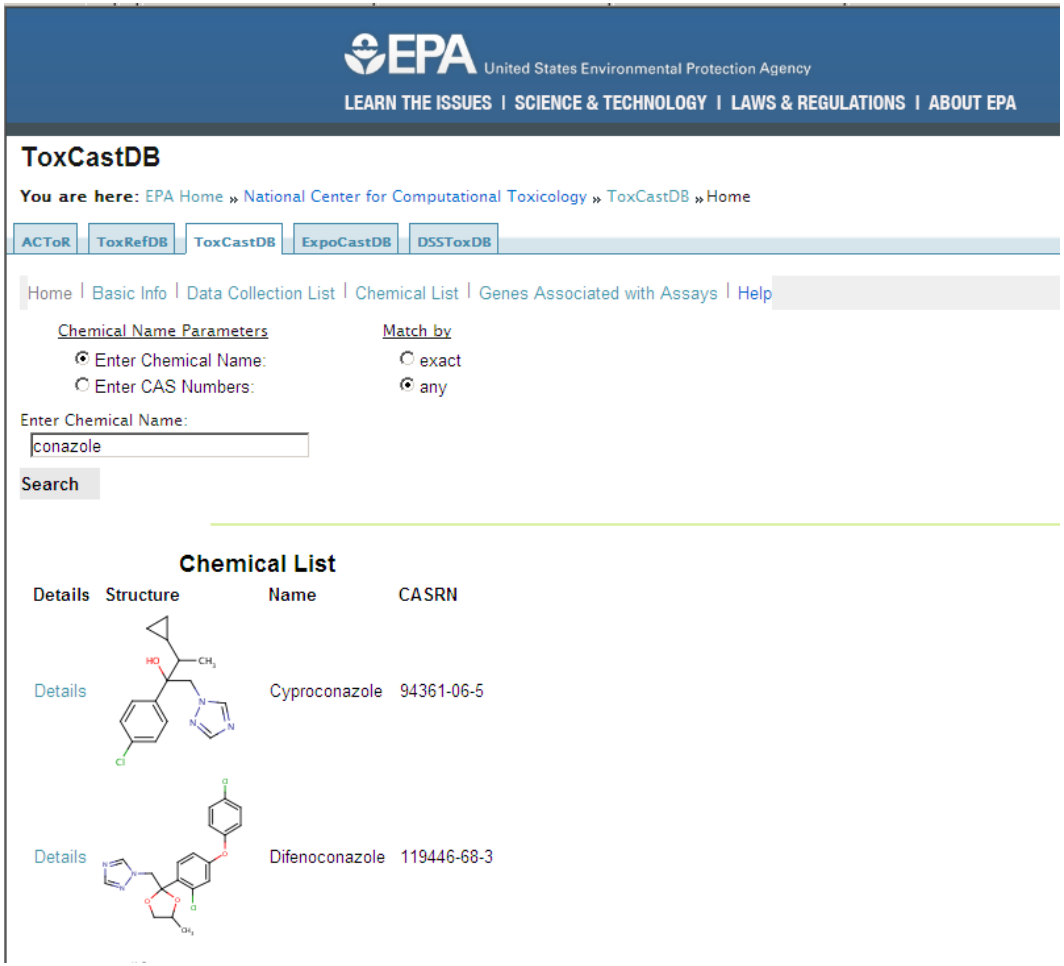
Not in current release

Multiple Assays per Endpoint



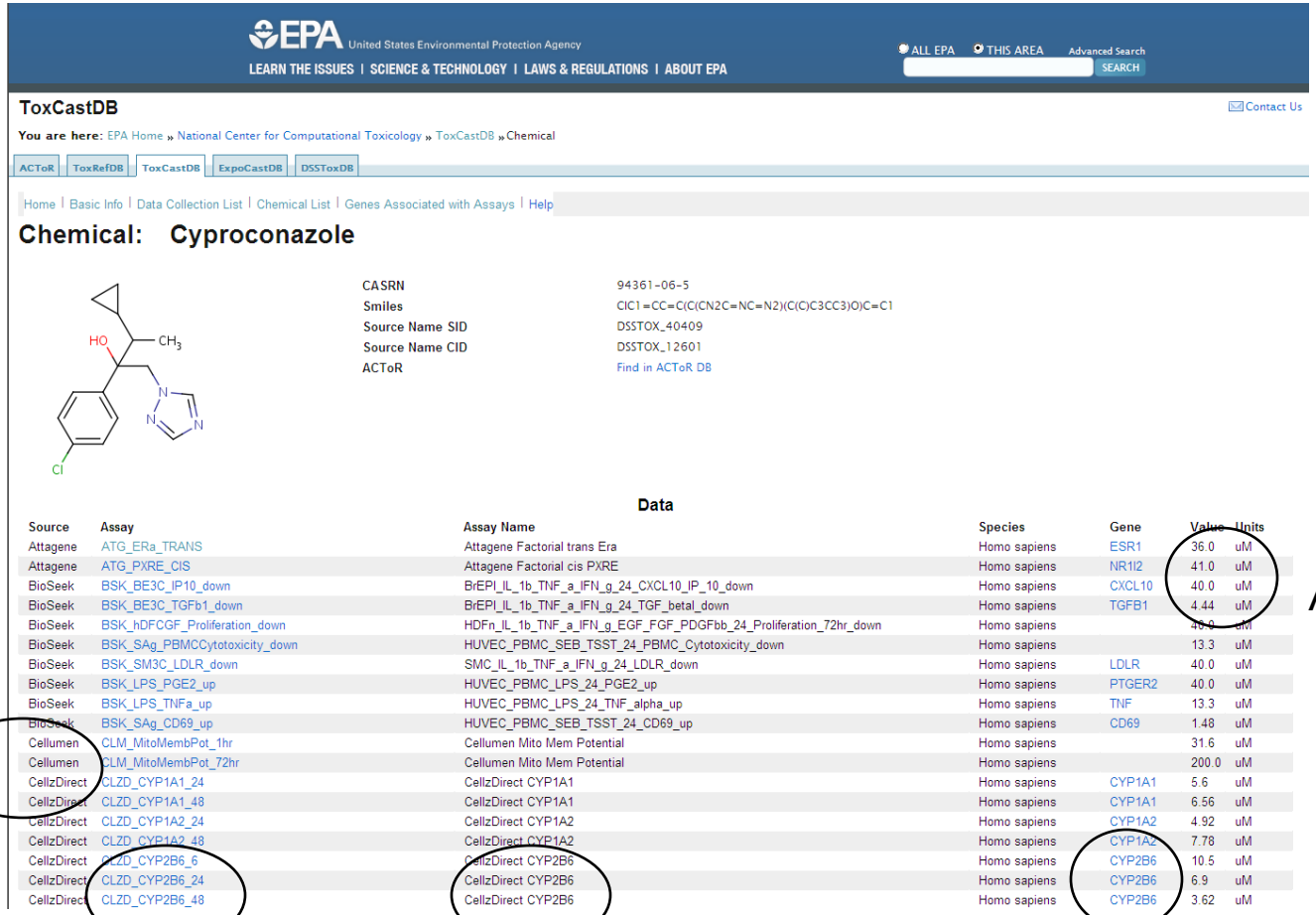
Browsing ToxCast / Tox21 Data

http://actor.epa.gov/actor/faces/ToxMiner/Home.jsp



Home page – search by name or CASRN

ToxCastDB Chemical Page



AC50 and units

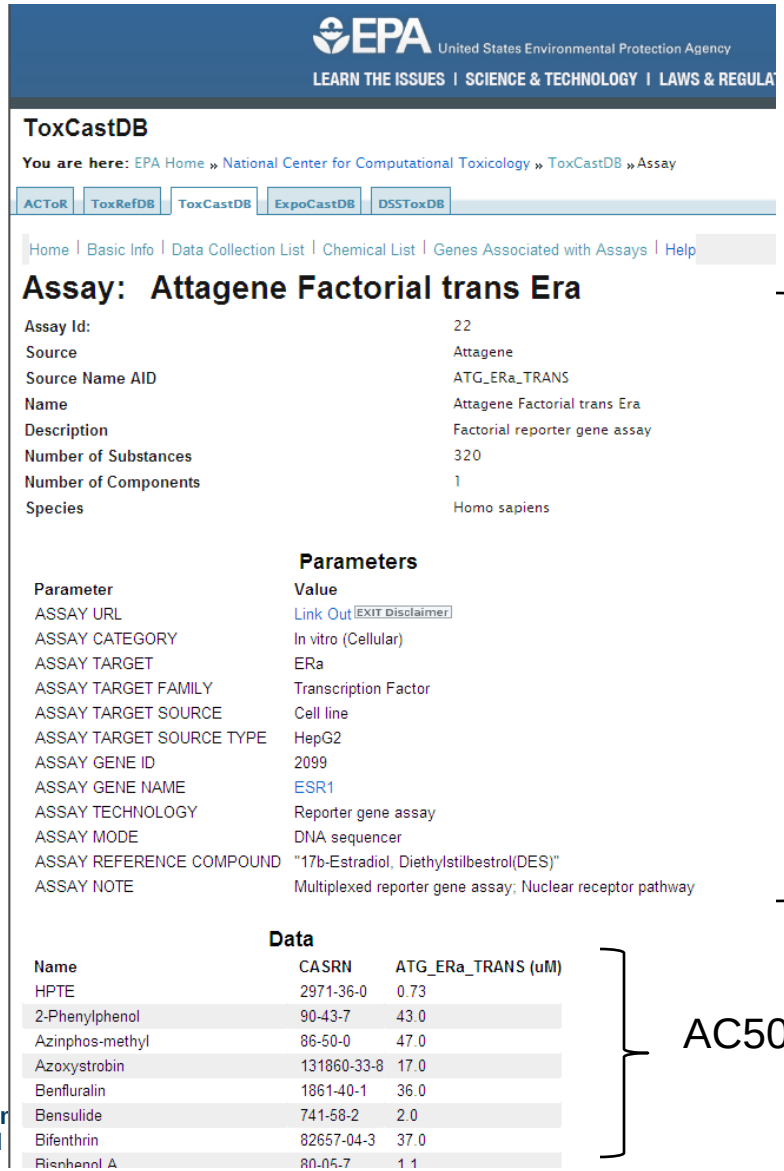
Assay Set

Assay Link

Assay Name

Gene Link to Entrez Gene

ToxCastDB Assay Page



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Assay: Attagene Factorial trans Era

Assay Id:	22
Source	Attagene
Source Name AID	ATG_ERa_TRANS
Name	Attagene Factorial trans Era
Description	Factorial reporter gene assay
Number of Substances	320
Number of Components	1
Species	Homo sapiens

Parameters

Parameter	Value
ASSAY URL	Link Out EXIT Disclaimer
ASSAY CATEGORY	In vitro (Cellular)
ASSAY TARGET	ERa
ASSAY TARGET FAMILY	Transcription Factor
ASSAY TARGET SOURCE	Cell line
ASSAY TARGET SOURCE TYPE	HepG2
ASSAY GENE ID	2099
ASSAY GENE NAME	ESR1
ASSAY TECHNOLOGY	Reporter gene assay
ASSAY MODE	DNA sequencer
ASSAY REFERENCE COMPOUND	"17b-Estradiol, Diethylstilbestrol(DES)"
ASSAY NOTE	Multiplexed reporter gene assay; Nuclear receptor pathway

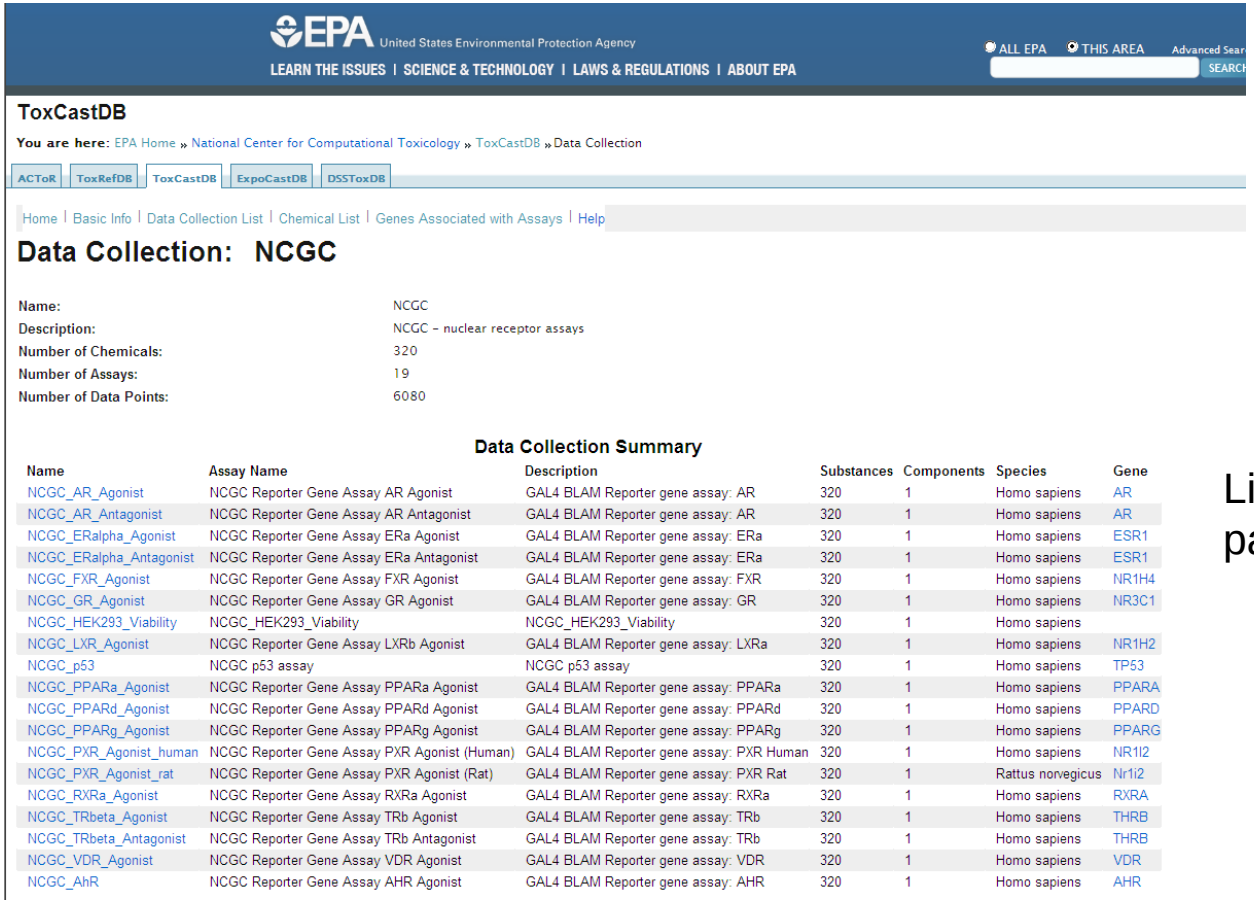
Data

Name	CASRN	ATG_ERa_TRANS (uM)
HPTE	2971-36-0	0.73
2-Phenylphenol	90-43-7	43.0
Azinphos-methyl	86-50-0	47.0
Azoxystrobin	131860-33-8	17.0
Benfluralin	1861-40-1	36.0
Bensulide	741-58-2	2.0
Bifenthrin	82657-04-3	37.0
Bisphenol A	80-05-7	1.1

Description of assay

AC50s for all positive chemicals

ToxCastDB Data Collection Page



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Data Collection: NCGC

Name: NCGC
Description: NCGC – nuclear receptor assays
Number of Chemicals: 320
Number of Assays: 19
Number of Data Points: 6080

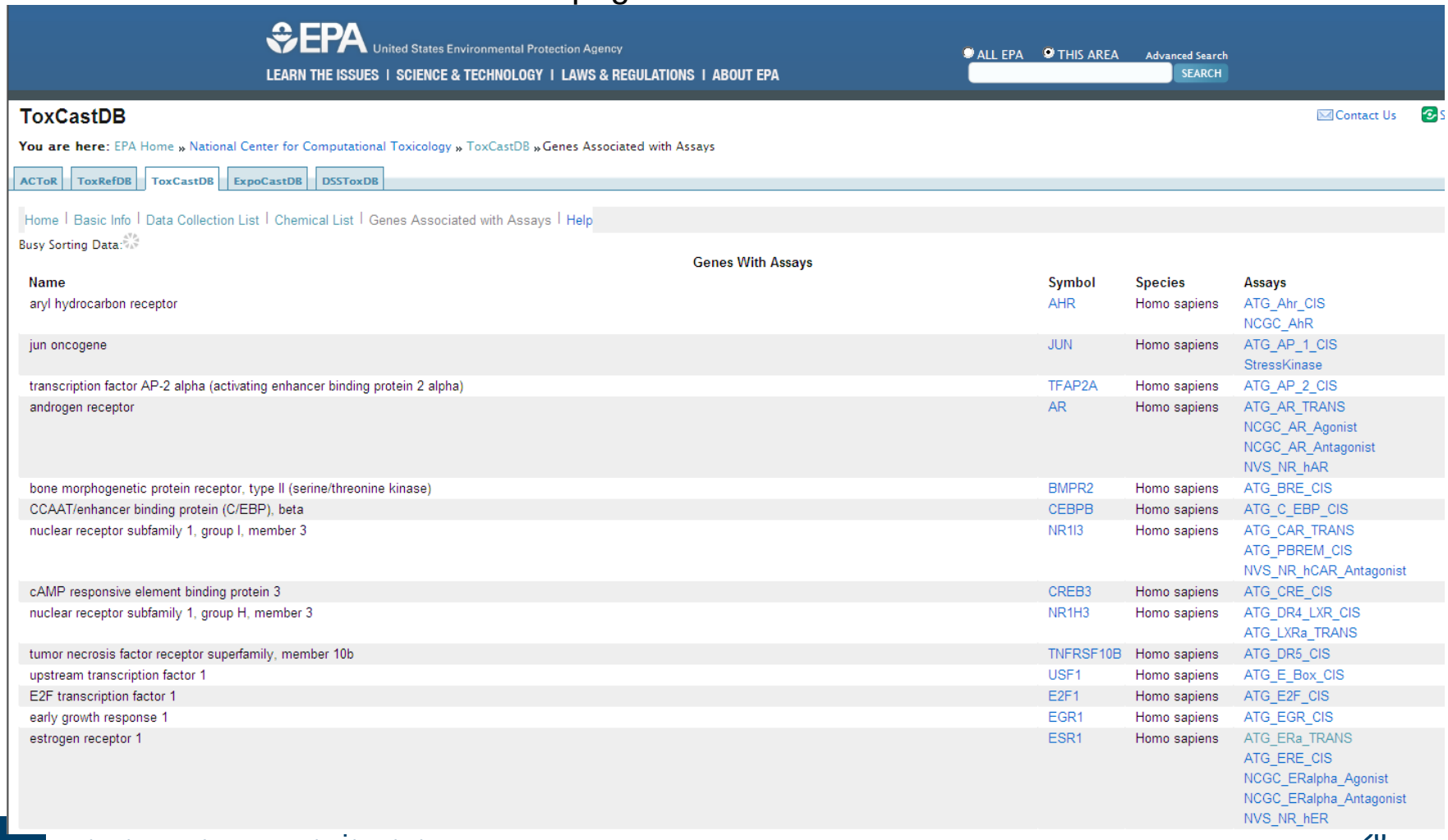
Data Collection Summary

Name	Assay Name	Description	Substances	Components	Species	Gene
NCGC_AR_Agonist	NCGC Reporter Gene Assay AR Agonist	GAL4 BLAM Reporter gene assay: AR	320	1	Homo sapiens	AR
NCGC_AR_Antagonist	NCGC Reporter Gene Assay AR Antagonist	GAL4 BLAM Reporter gene assay: AR	320	1	Homo sapiens	AR
NCGC_ERalpha_Agonist	NCGC Reporter Gene Assay ERa Agonist	GAL4 BLAM Reporter gene assay: ERa	320	1	Homo sapiens	ESR1
NCGC_ERalpha_Antagonist	NCGC Reporter Gene Assay ERa Antagonist	GAL4 BLAM Reporter gene assay: ERa	320	1	Homo sapiens	ESR1
NCGC_FXR_Agonist	NCGC Reporter Gene Assay FXR Agonist	GAL4 BLAM Reporter gene assay: FXR	320	1	Homo sapiens	NR1H4
NCGC_GR_Agonist	NCGC Reporter Gene Assay GR Agonist	GAL4 BLAM Reporter gene assay: GR	320	1	Homo sapiens	NR3C1
NCGC_HEK293_Viability	NCGC_HEK293_Viability	NCGC_HEK293_Viability	320	1	Homo sapiens	
NCGC_LXR_Agonist	NCGC Reporter Gene Assay LXRb Agonist	GAL4 BLAM Reporter gene assay: LXRa	320	1	Homo sapiens	NR1H2
NCGC_p53	NCGC p53 assay	NCGC p53 assay	320	1	Homo sapiens	TP53
NCGC_PPARa_Agonist	NCGC Reporter Gene Assay PPARa Agonist	GAL4 BLAM Reporter gene assay: PPARa	320	1	Homo sapiens	PPARA
NCGC_PPARd_Agonist	NCGC Reporter Gene Assay PPARd Agonist	GAL4 BLAM Reporter gene assay: PPARd	320	1	Homo sapiens	PPARD
NCGC_PPARg_Agonist	NCGC Reporter Gene Assay PPARg Agonist	GAL4 BLAM Reporter gene assay: PPARg	320	1	Homo sapiens	PPARG
NCGC_PXR_Agonist_human	NCGC Reporter Gene Assay PXR Agonist (Human)	GAL4 BLAM Reporter gene assay: PXR Human	320	1	Homo sapiens	NR1I2
NCGC_PXR_Agonist_rat	NCGC Reporter Gene Assay PXR Agonist (Rat)	GAL4 BLAM Reporter gene assay: PXR Rat	320	1	Rattus norvegicus	Nr1i2
NCGC_RXRa_Agonist	NCGC Reporter Gene Assay RXRa Agonist	GAL4 BLAM Reporter gene assay: RXRa	320	1	Homo sapiens	RXRA
NCGC_TRbeta_Agonist	NCGC Reporter Gene Assay TRb Agonist	GAL4 BLAM Reporter gene assay: TRb	320	1	Homo sapiens	THRB
NCGC_TRbeta_Antagonist	NCGC Reporter Gene Assay TRb Antagonist	GAL4 BLAM Reporter gene assay: TRb	320	1	Homo sapiens	THRB
NCGC_VDR_Agonist	NCGC Reporter Gene Assay VDR Agonist	GAL4 BLAM Reporter gene assay: VDR	320	1	Homo sapiens	VDR
NCGC_AhR	NCGC Reporter Gene Assay AHR Agonist	GAL4 BLAM Reporter gene assay: AHR	320	1	Homo sapiens	AHR

List of all assays from a particular data set

Genes associated with assays

Lists all assays associated with each gene
NOTE: this page is slow to load



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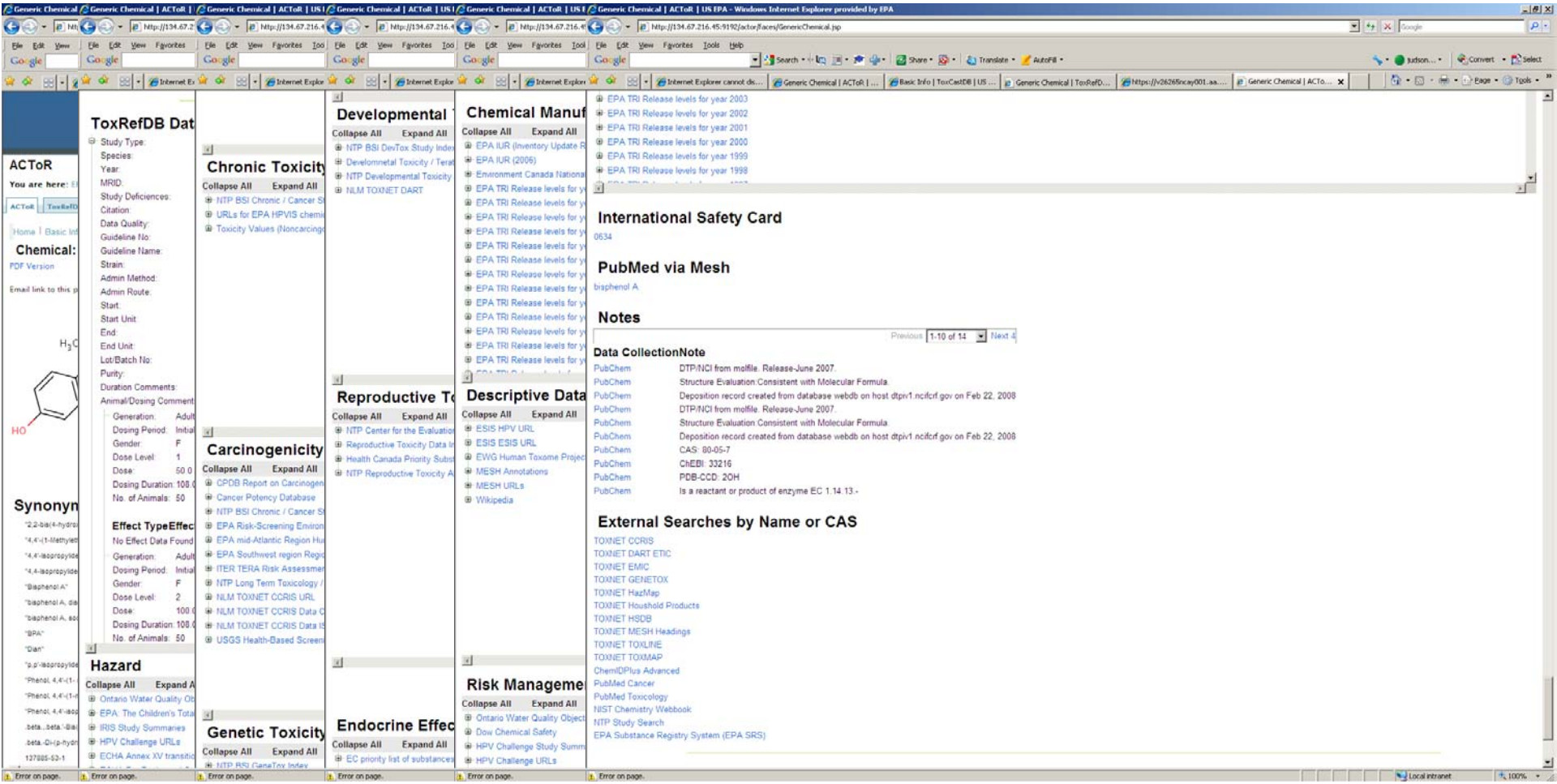
Busy Sorting Data:

Genes With Assays

Name	Symbol	Species	Assays
aryl hydrocarbon receptor	AHR	Homo sapiens	ATG_Ahr_CIS NCGC_AhR
jun oncogene	JUN	Homo sapiens	ATG_AP_1_CIS StressKinase
transcription factor AP-2 alpha (activating enhancer binding protein 2 alpha)	TFAP2A	Homo sapiens	ATG_AP_2_CIS
androgen receptor	AR	Homo sapiens	ATG_AR_TRANS NCGC_AR_Agonist NCGC_AR_Antagonist NVS_NR_hAR
bone morphogenetic protein receptor, type II (serine/threonine kinase)	BMPR2	Homo sapiens	ATG_BRE_CIS
CCAAT/enhancer binding protein (C/EBP), beta	CEBPB	Homo sapiens	ATG_C_EBP_CIS
nuclear receptor subfamily 1, group I, member 3	NR1I3	Homo sapiens	ATG_CAR_TRANS ATG_PBREM_CIS NVS_NR_hCAR_Antagonist
cAMP responsive element binding protein 3	CREB3	Homo sapiens	ATG_CRE_CIS
nuclear receptor subfamily 1, group H, member 3	NR1H3	Homo sapiens	ATG_DR4_LXR_CIS ATG_LXRa_TRANS
tumor necrosis factor receptor superfamily, member 10b	TNFRSF10B	Homo sapiens	ATG_DR5_CIS
upstream transcription factor 1	USF1	Homo sapiens	ATG_E_Box_CIS
E2F transcription factor 1	E2F1	Homo sapiens	ATG_E2F_CIS
early growth response 1	EGR1	Homo sapiens	ATG_EGR_CIS
estrogen receptor 1	ESR1	Homo sapiens	ATG_ERa_TRANS ATG_ERE_CIS NCGC_ERalpha_Agonist NCGC_ERalpha_Antagonist NVS_NR_hER

ACToR Chemical Page – many sources of data



Coming Soon

- Mouse Embryonic Stem Cell Data (With Sid Hunter, EPA NHEERL)
- RTK Data – Reverse Toxicokinetics (with R. Thomas, Hamner)
- ExpoCastDB (With collaborators in EPA NERL)

Outline: ToxCast Data Sets Overview

- Data Sets: Bio-centric View
  - Assay overview
  - How were the data calculated?
  - How did the replicates perform?
  - Example results
  - What did the assay measure?
  - Potential assay artifacts

ToxCast Phase I Data Sets

- Biochemical
  - Novascreen
- Cell-Based
  - ACEA
  - Attagene
  - BioSeek
  - Cellumen
  - CellzDirect
  - Gentronix
  - NCGC
  - Solidus

Novascreen: 239 Biochemical Assays (Abstract 59)

- Protein super-families
  - GPCR
  - Kinase
  - Phosphatase
  - Protease
  - Ion channel
  - Nuclear receptor
  - Other enzyme
  - CYP P450 inhibition

- Various formats:
  - Radioligand receptor binding
  - Fluorescent receptor binding
  - Fluorescent enzyme substrate-intensity quench
  - Fluorescent enzyme substrate-mobility shift
- Initial screening:
  - 25 μM in duplicate
  - 10 μM in duplicate (CYPs)
- Normalize data to assay window
  - % of control activity (central reference – scalar reference)

Novascreen: What do the assays measure?

- Mainly direct effects of chemical on target protein
  - Enzyme activity
  - Ligand binding
- False positives:
  - Fluorescent compounds—fluorescing and quenching
  - Reactive compounds/covalent modification of target
  - Physical effects—colloid aggregation of target
  - Operational
- False negatives:
  - Solubility
  - Inappropriate assay conditions
  - Operational
  - Target protein not physiological
  - Lack of biotransformation

Data Correction Algorithm Examples (Additive)

normalized

AChE

	1	2	3	4	5	6	7	8	9	10	11	12
A	-3	3	-29	-100	-98	-100	-68	-68	-89	-89	-98	-98
B	-4	7	-29	-12	-10	-12	-27	-22	-30	-30	-25	-16
C	8	2	-23	-23	-19	-26	-25	-26	-26	-28	-25	-13
D	-14	1	-17	-21	-23	-25	-18	-26	-26	-27	-25	-17
E	-11	-5	-23	-25	-16	-20	-26	-22	-27	-30	-26	-13
F	-16	0	-7	-7	-17	-21	-22	-24	-29	-33	-19	-17
G	-5	4	-32	-18	-25	-17	-25	-28	-25	-32	-20	-13
H	-85	-78	-22	-22	2	-14	-17	-17	NaN	NaN	NaN	NaN

Caspase 10

	1	2	3	4	5	6	7	8	9	10	11	12
A	25	0	-3	-97	-100	-101	-35	-33	-76	-80	-100	-99
B	9	-17	-16	-23	-28	-17	-13	-16	-22	-15	-22	-3
C	3	-22	-10	-27	-24	-20	-39	-31	-24	-28	-26	-6
D	15	-22	-15	-27	-23	-27	-13	-15	-17	-18	-20	-3
E	3	-16	-16	-17	-18	-7	-6	-13	2	5	-17	12
F	-11	-34	-18	-26	-29	-21	-22	-19	-18	-19	-25	0
G	-23	-30	-27	-24	-17	-6	-8	-11	-22	-12	-9	10
H	-15	-28	-17	-20	-14	-28	-5	4	-19	-4	-18	5

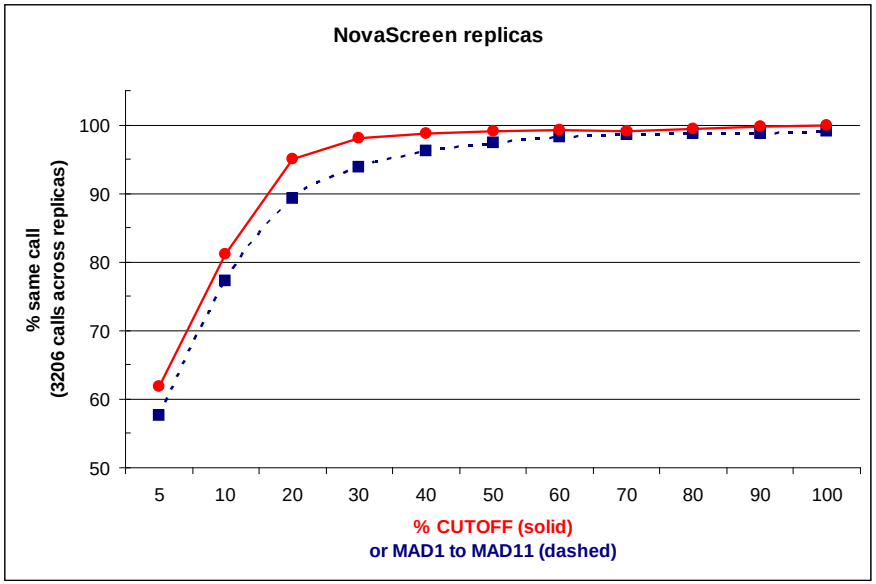
Normalized & corrected

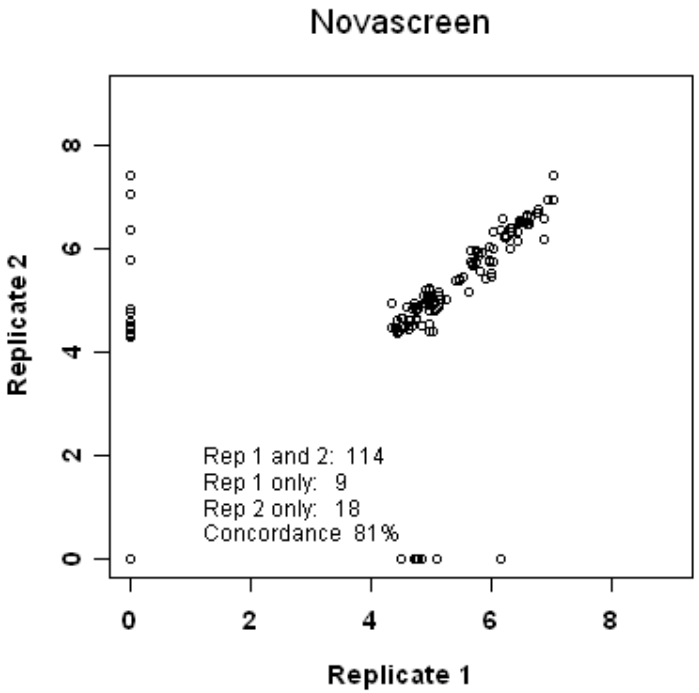
	1	2	3	4	5	6	7	8	9	10	11	12
A	-1	1	-19	-100	-98	-100	-67	-67	-89	-89	-98	-98
B	0	7	-20	-10	-2	0	2	9	-15	-16	-9	-4
C	10	10	-3	-3	0	-6	-2	-5	-16	-18	-11	-1
D	4	14	7	2	2	-5	6	0	-13	-11	-9	-3
E	7	13	4	0	7	4	-2	2	-15	-18	-10	-1
F	5	22	16	16	9	6	3	3	-15	-17	-3	-2
G	12	23	-4	6	1	5	3	0	-11	-16	-1	4
H	-52	-44	7	9	20	8	4	5	NaN	NaN	NaN	NaN

	1	2	3	4	5	6	7	8	9	10	11	12
A	23	0	-3	-97	-100	-101	-36	-34	-76	-81	-100	-99
B	13	6	7	0	-6	-3	3	2	-6	1	-5	0
C	6	-1	10	-7	-4	-5	-21	-13	-5	-10	-7	-5
D	18	0	2	-7	0	-7	6	4	0	-1	-4	1
E	1	1	2	-3	-5	-2	7	0	14	17	-6	9
F	-9	-12	4	-3	-11	-9	-4	-1	-7	-4	-11	-1
G	-17	-5	-5	-4	-1	-1	13	6	-2	3	5	8
H	-9	-11	2	0	4	-14	9	15	-4	10	-7	8

Novascreen Concentration-Response Testing

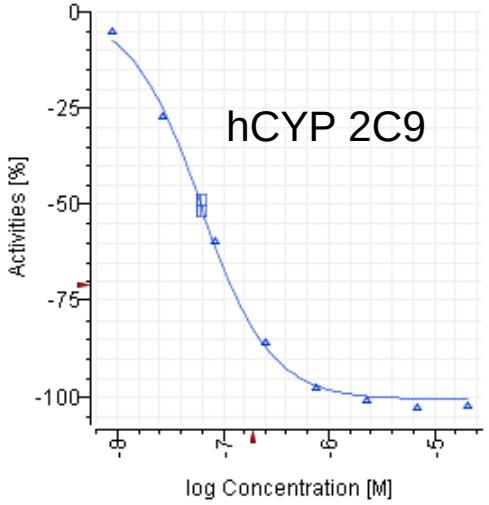
- Retest actives:
  - Median absolute deviation (MAD) median I*x-xmed*I two MADs or 30% activity
  - 8 conc/3-fold serial dilutions
    - 50 μM high conc
    - 25 μM high conc for CYPs
- Normalize to assay window
- Fit % Activity data to 3- or 4-parameter Hill function
  - Sometimes had to fix top or bottom of curve
  - Did not extrapolate beyond testing range
  - Manual or automated removal of obvious outliers

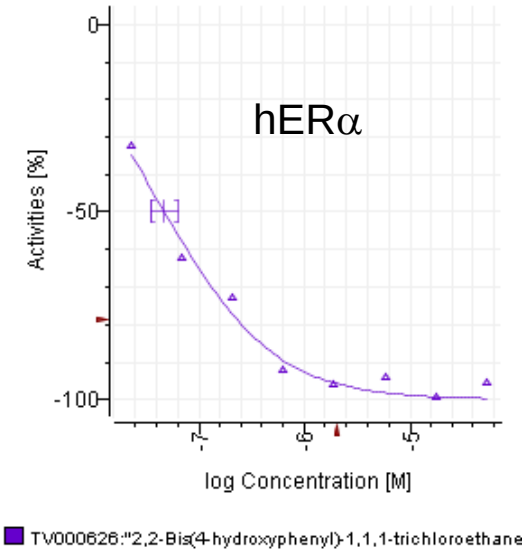


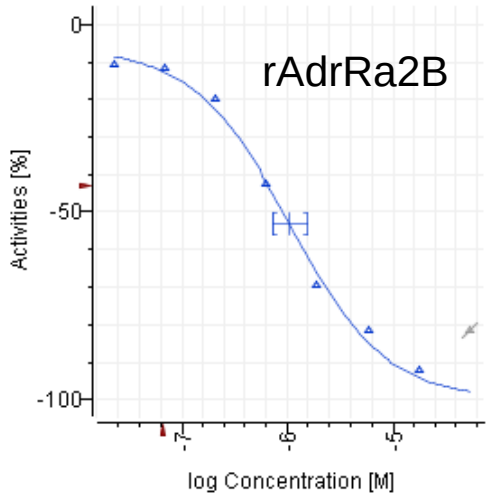


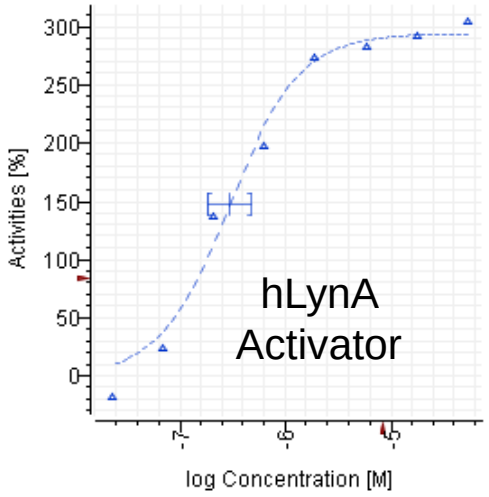
Novascreen: Example Curve Fits

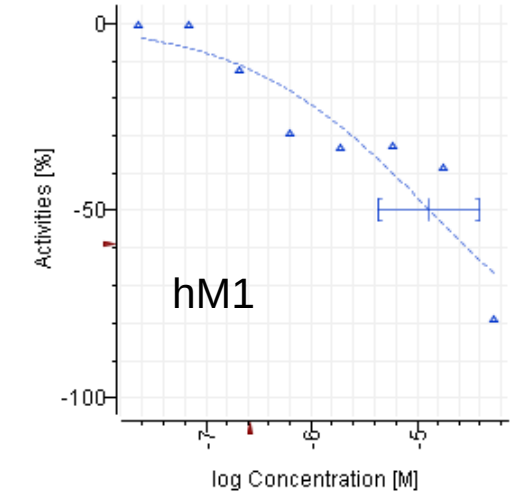
United States Environmental Protection Agency

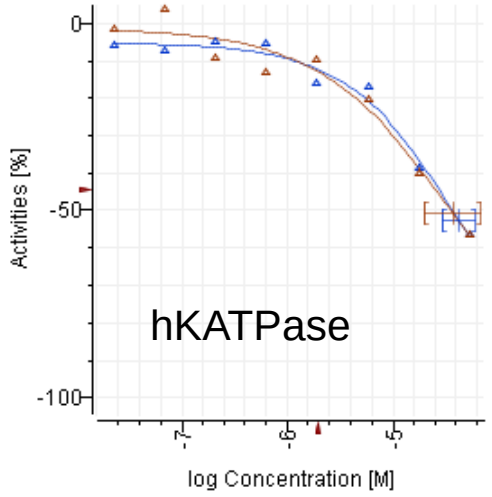








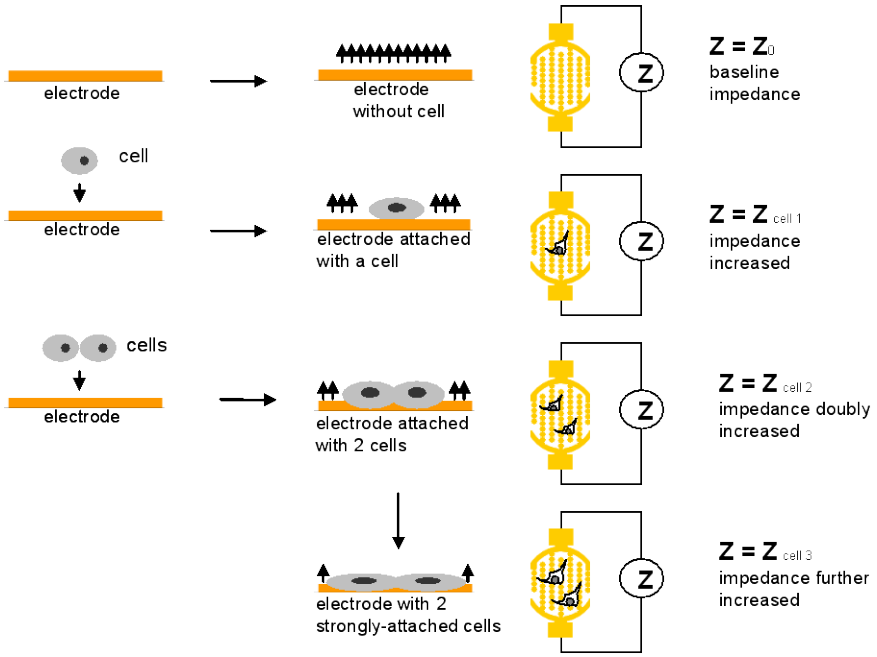




ACEA: Real Time Cell Growth Kinetics

United States Environmental Protection Agency

- Cytotoxicity with potential mechanistic interpretation
- Human A549 lung carcinoma cell line
  - ACEA experience with line
  - Reference compound effects
- Concentration-response testing
  - 8 conc/3-fold serial dilutions
  - Duplicate wells
- Real-time measuremens during exposure (0-72 hr)
- IC50 and LELs calculated



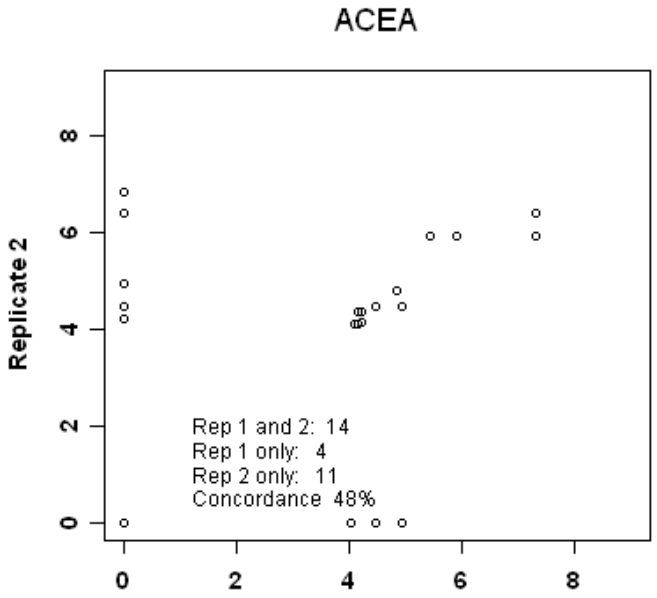
ACEA: What is measured?

- General cytotoxicity in transformed cell line
- False positives
  - No obvious
  - Operational
- False negatives
  - Operational error
  - Solubility
  - Lack of appropriate toxicity targets
  - Lack of biotransformation

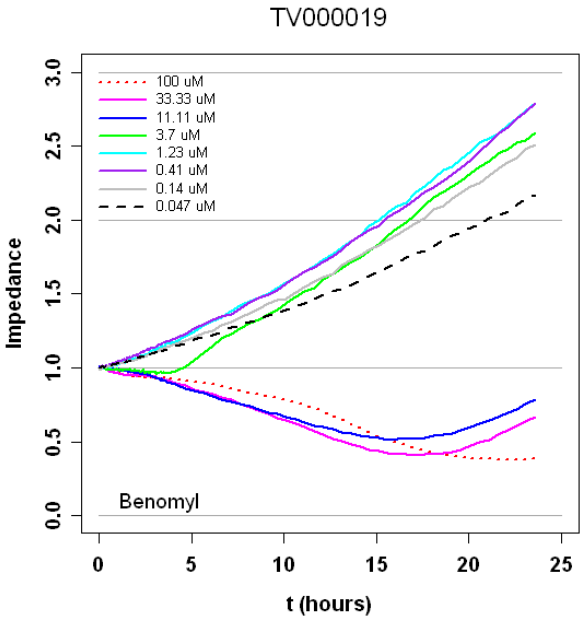
ACEA: Data examples

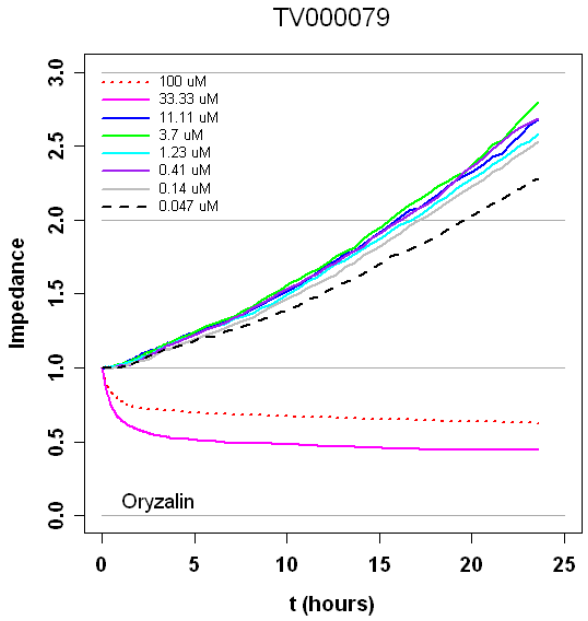
United States Environmental Protection Agency

Replicate Analysis:



Example Plots:





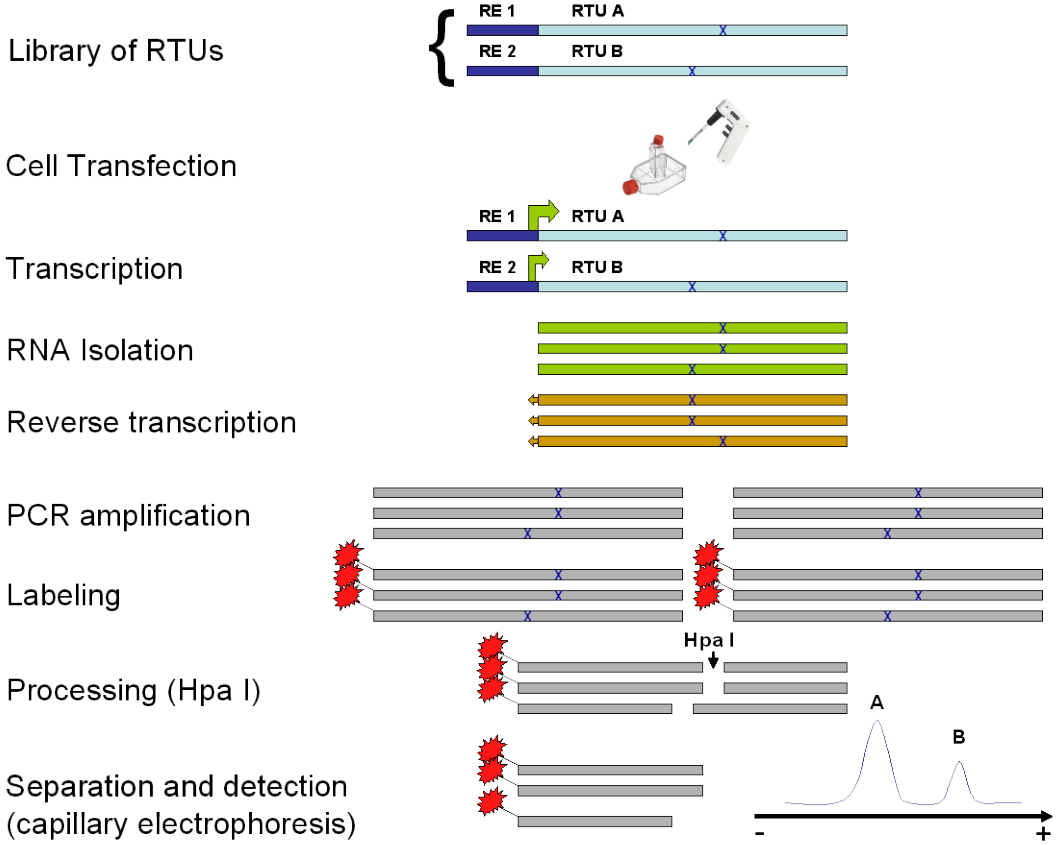
Attagene Multiplexed Transcription Factor Assays

- Modulation of TF activity in human hepatoma HepG2 cells
- Multiplexed reporter gene assay
  - *cis* 52 assays (response element driving reporter)
  - *trans* 29 assays (GAL4-NR_LBD driving reporter) “ligand detection”
- IC50 for cytotoxicity measured first in HepG2
- High concentration either 100 μM or 1/3 calculated IC50 for cytotoxicity
- Seven concentrations, 3-fold serial dilutions, 24 hr exposure
- Cells harvested, RNA isolated, processed for reporter gene quantitation
- LEL provided in data set

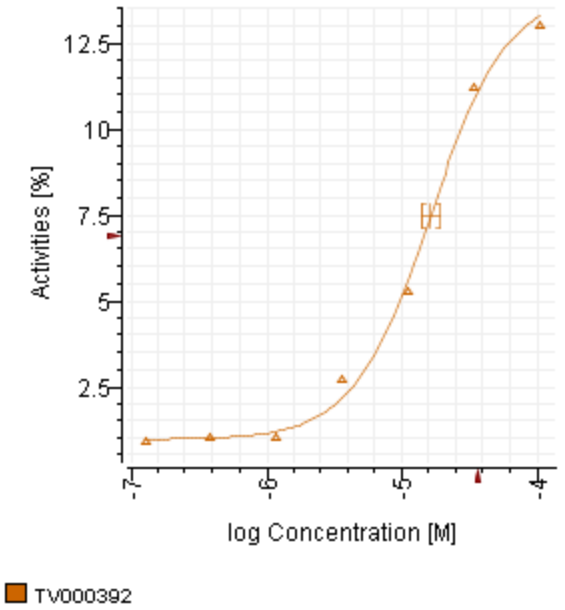
Attagene Technology

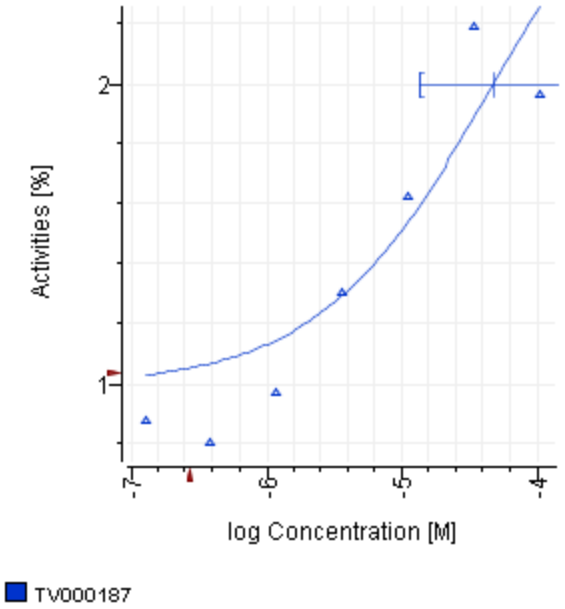
United States Environmental Protection Agency

Multiplexed Reporter Gene Assay



Cis: AhR





Attagene: What Is Being Measured?

- cis Assays
  - Up/down regulation of endogenous transcription factor activity in transformed cell line
  - False positives
    - General cytotoxic response resulting in non-specific transcriptional activity
    - Promiscuity of response elements
    - Statistical, not biologically, significant response
    - Operational
  - False negatives
    - Solubility
    - Cytotoxicity
    - Operational
    - Lack of endogenous machinery
    - Lack of biotransformation
- trans Assays
  - NR agonist activity
  - False positives
    - General cytotoxic response resulting in non-specific transcriptional activity
    - Statistical, not biologically, significant response
    - Operational
  - False negatives
    - Solubility
    - Cytotoxicity
    - Operational
    - Lack of endogenous machinery
    - Lack of biotransformatioin

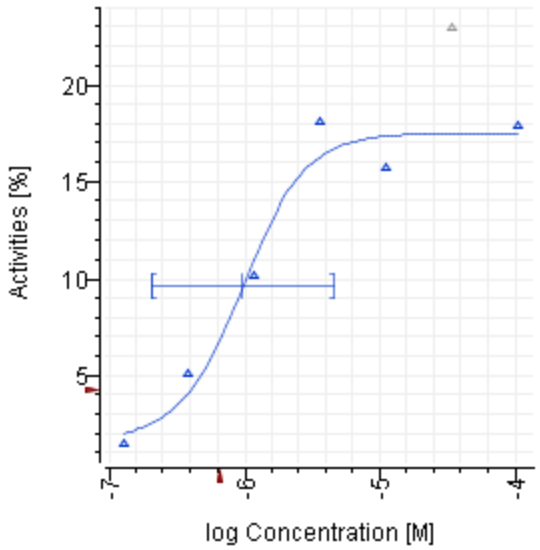
Attagene: corresponding *cis* and *trans* assays

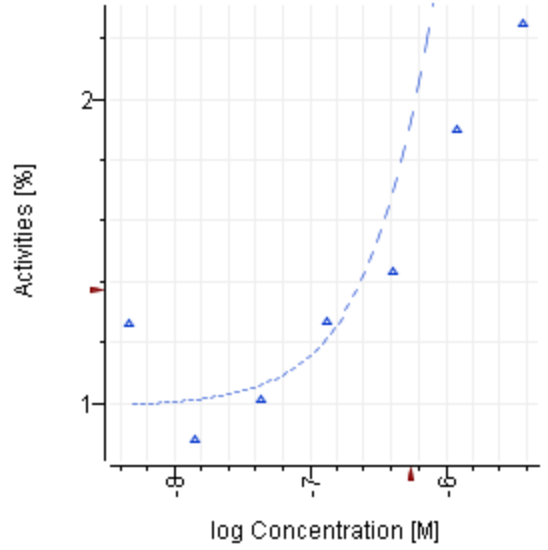
United States Environmental Protection Agency

Bisphenol A

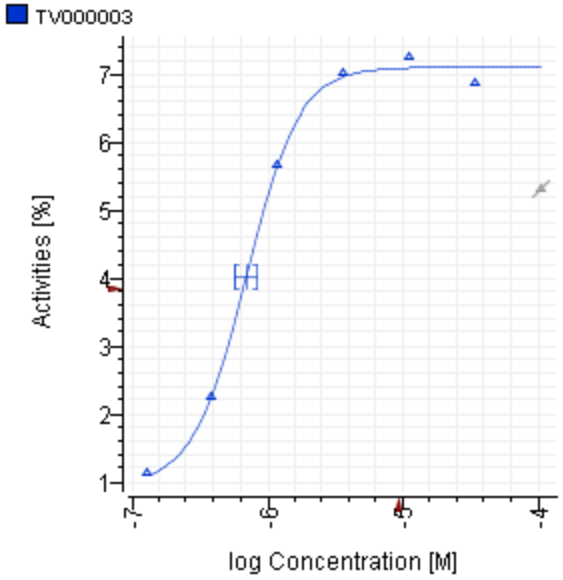
HPTE

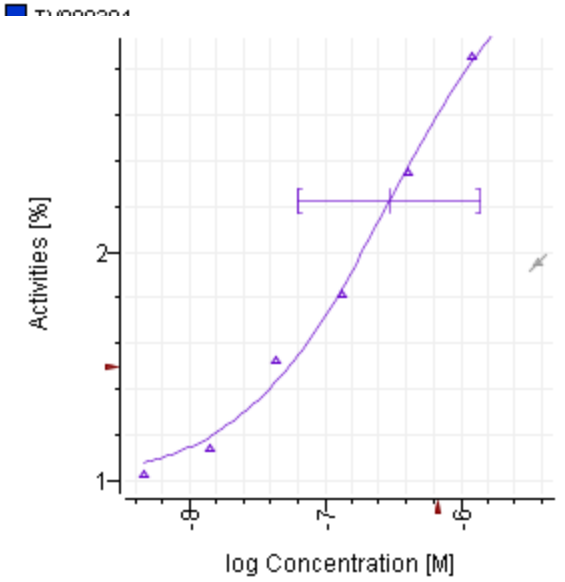
***trans*: ERa**





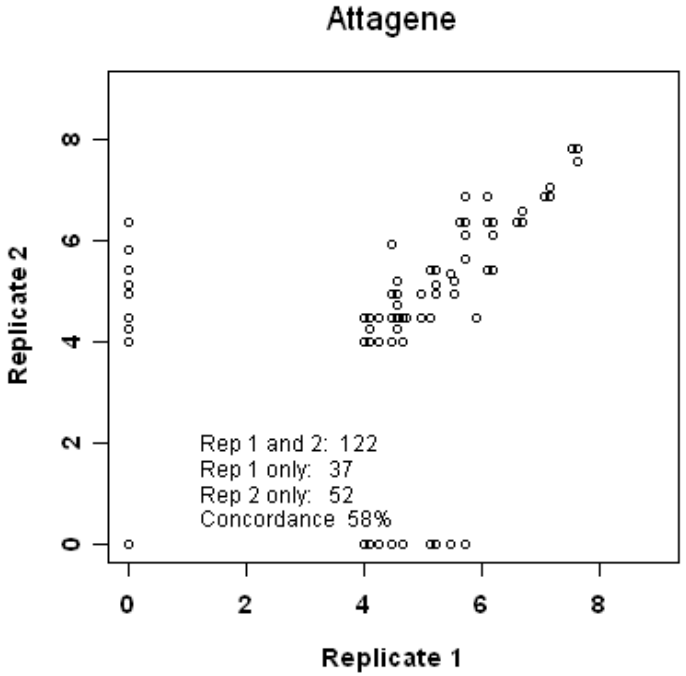
***cis*: ERE**

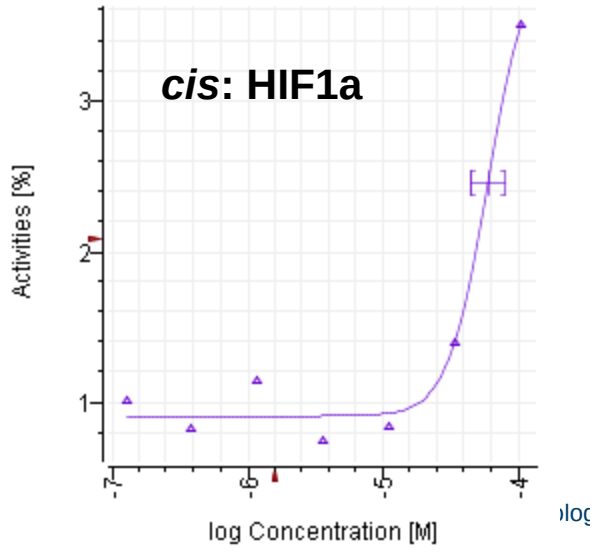


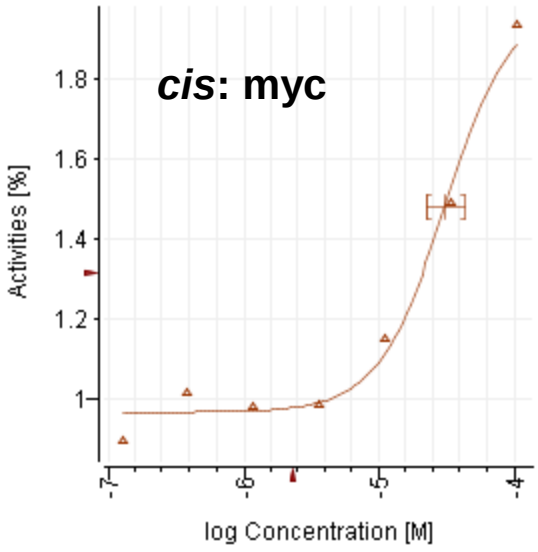


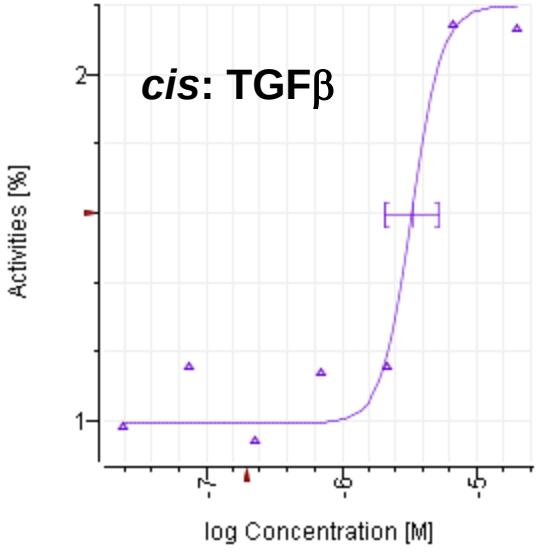
Attagene: Data calculation challenges

- No positive reference compound for each endpoint
- Responses, especially for *cis* assays, tended to be montonic so potency value difficult to derive
- Biological vs statistical significance for LEL









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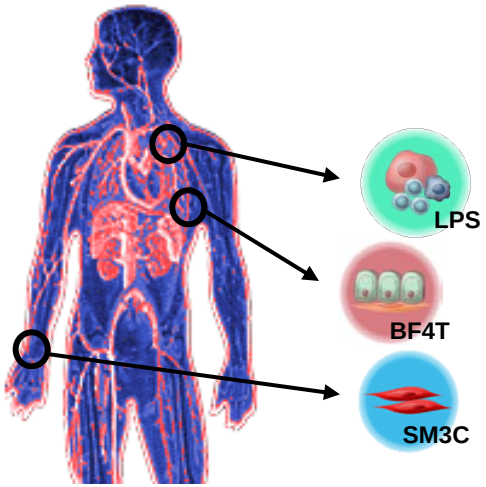
BioSeek: BioMAP® Technology Platform (Abstract 24)

United States Environmental Protection Agency

Assays

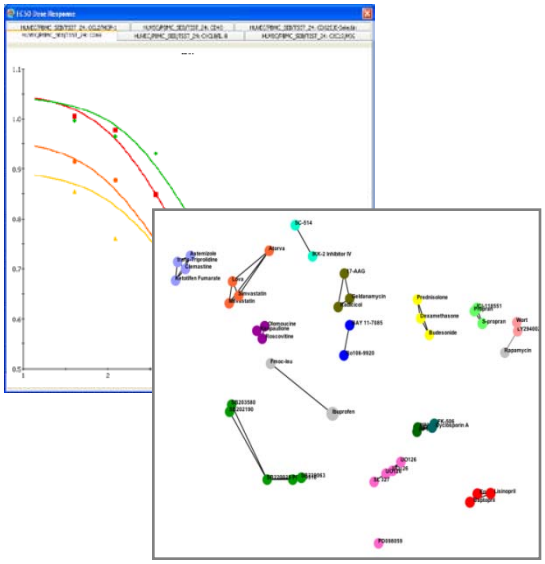


Human primary cells
Disease-like culture conditions

Profile Database

Biological responses to drugs and stored in the database

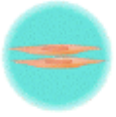
Informatics



Specialized informatics tools are used to mine and analyze biological data

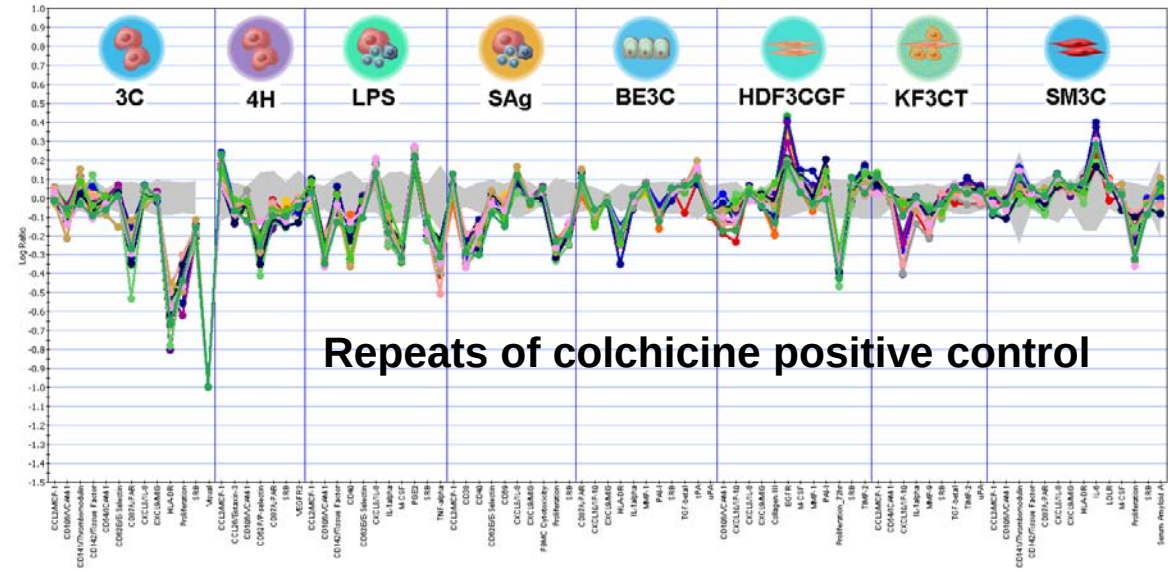
Primary Human Cell-Based Assay Platform for Human Pharmacology

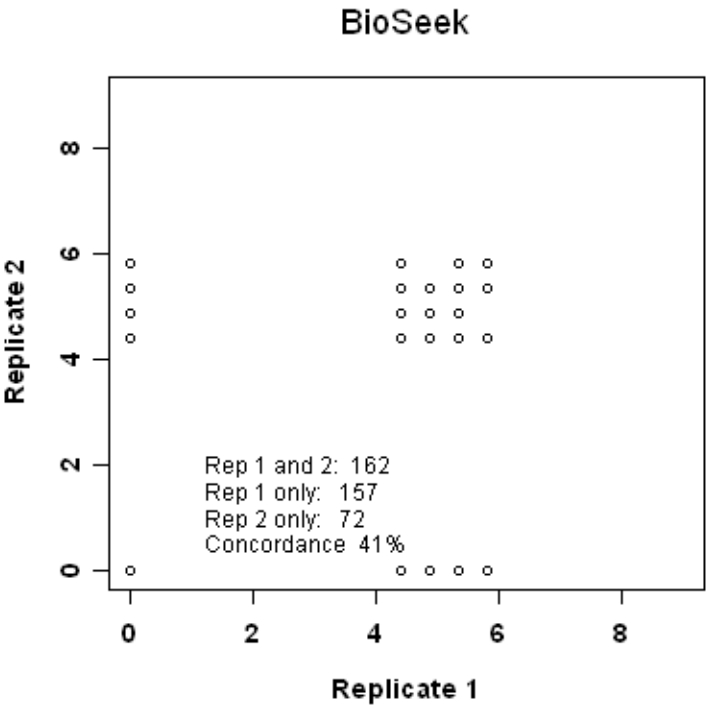
BioSeek Assays Tested Against ToxCast_320

System		Cell Types	Environment	Readouts
3C		Endothelial cells	IL-1β+TNF-α+IFN-γ	MCP-1, VCAM-1, ICAM-1, Thrombomodulin, Tissue Factor, E-selectin, uPAR, IL-8, MIG, HLA-DR, Prolif., Vis., SRB (13)
4H		Endothelial cells	IL-4+histamine	VEGFRII, P-selectin, VCAM-1, uPAR, Eotaxin-3, MCP-1, SRB (7)
LPS		Peripheral Blood Mononuclear Cells + Endothelial cells	TLR4	CD40, VCAM-1,Tissue Factor, MCP-1, E-selectin, IL-1a, IL-8, M-CSF, TNF-a, PGE2, SRB (11)
SAg		Peripheral Blood Mononuclear Cells + Endothelial cells	TCR	MCP-1, CD38, CD40, CD69, E-selectin, IL-8, MIG, PBMC Cytotox., SRB, Proliferation (10)
BE3C		Bronchial epithelial cells	IL-1β+TNF-α+IFN-γ	uPAR, IP-10, MIG, HLA-DR, IL-1a, MMP-1, PAI-1, SRB, TGF-b1, tPA, uPA (11)
HDF3CGF		Fibroblasts	IL-1β+TNF-α+IFN-γ +bFGF+EGF+PDGF-BB	VCAM-1, IP-10, IL-8, MIG, Collagen III, M-CSF, MMP-1, PAI-1, Proliferation, TIMP-1, EGFR, SRB (12)
KF3CT		Keratinocytes + Fibroblasts	IL-1β+TNF-α+IFN-γ +TGF-β	MCP-1, ICAM-1, IP-10, IL-1a, MMP-9, TGF-b1, TIMP-2, uPA, SRB (9)
SM3C		Vascular smooth muscle cells	IL-1β+TNF-α+IFN-γ	MCP-1, VCAM-1, Thrombomodulin, Tissue Factor, IL-6, LDLR, SAA, uPAR, IL-8, MIG, HLA-DR, M-CSF, Prolif., SRB (14)

BioSeek Assays

- Chemicals tested at 4 concentrations: 40, 13.3, 4.4, 1.5 μM, single well
- Exposure started 1 hr before stimulation of cell signaling pathways
- Following 24 hr exposure, endpoints measured by ELISA (also Alamar blue, SRB staining, and microscopy)
- Data calculated vs 6 DMSO controls as fold-change
- Up and down regulation distinguished
- LELs determined



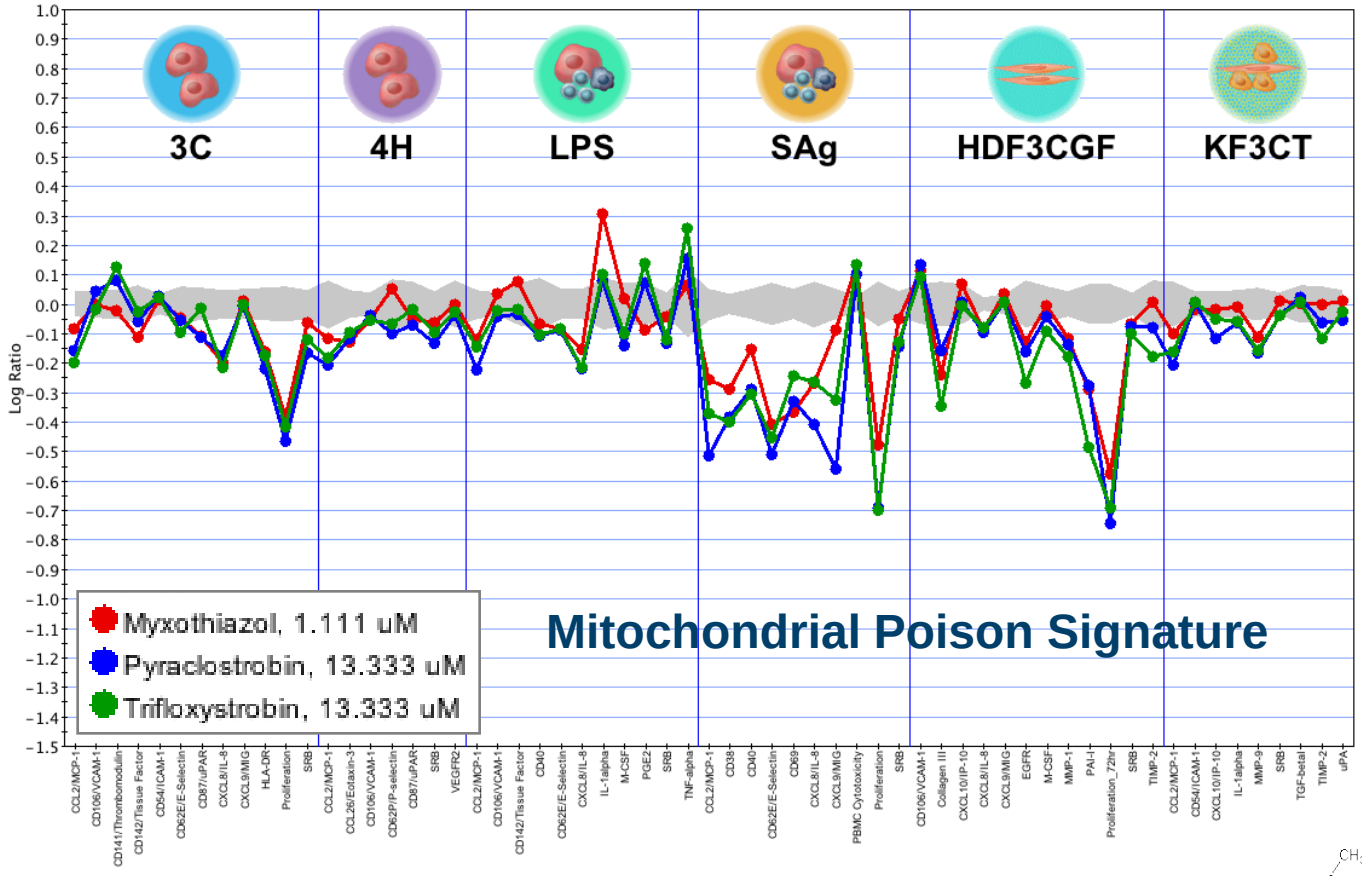


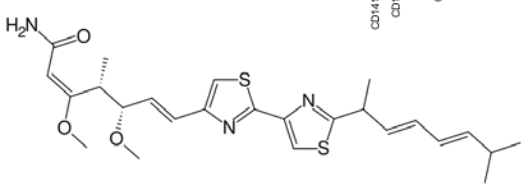
BioSeek Assays: What is Being Measured?

- Effects of chemicals on signaling pathways in primary human cells
- False positives
  - Cytotoxicity (down-regulation in particular)
  - Statistical vs biological significance
  - Operational
- False negatives
  - Solubility
  - Lack of biotransformation
  - Cytotoxicity
  - Operational

BioSeek MOA Signatures

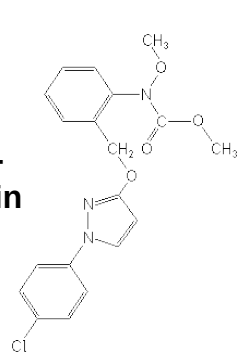
Defined by reference cmpd behavior in assays used



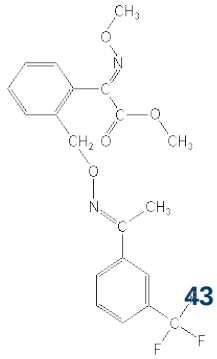


Myxothiazol (antibiotic) is an inhibitor of coenzyme Q - cytochrome c reductase (aka complex III). It prevents electron transfer from Fe-S center to ubiquinone in the electron transport chain.

Strobilurin fungicides: MOA-- blocks electron transfer within respiratory chain



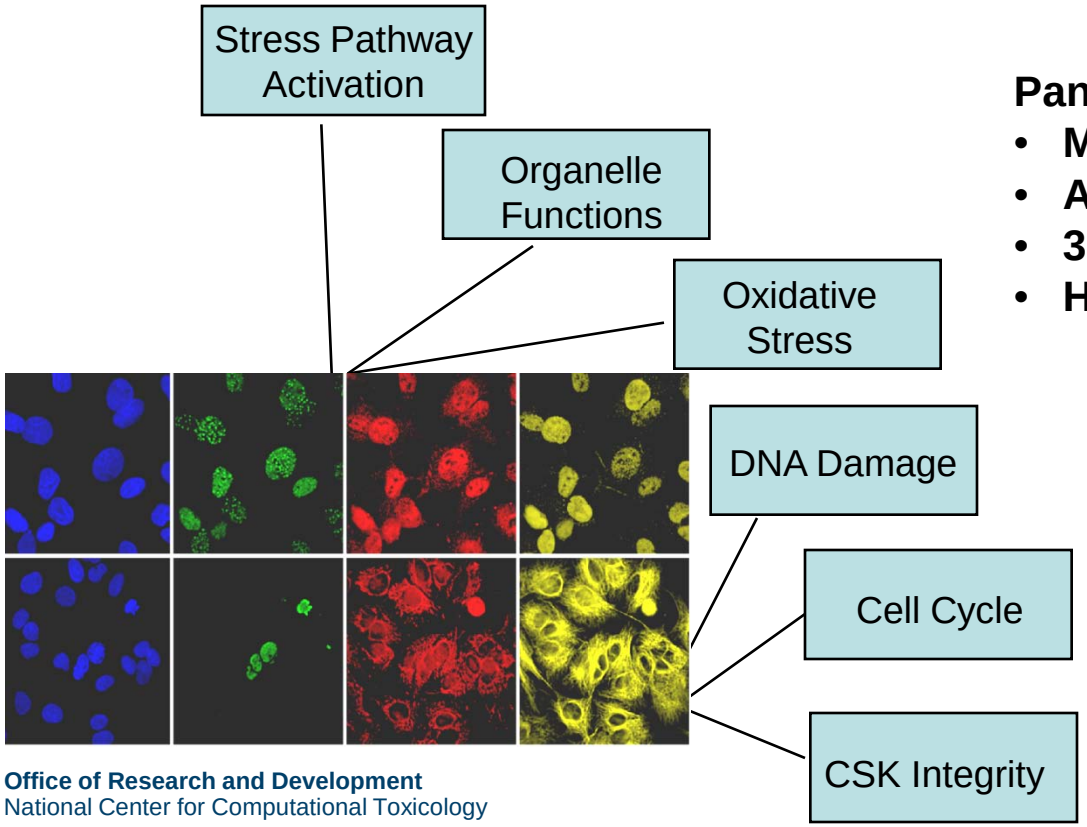
Pyraclostrobin



Trifloxystrobin

Cellumen: High-Content Screening of Cellular Phenotypic Toxicity Parameters (Abstract 38)

- Technology: automated fluorescent microscopy
- Objective: Determine effects of chemicals on toxicity biomarkers in a cell culture of HepG2 and primary rat hepatocytes



Panel 1 design*:

- **Multiple mechanisms of toxicity**
- **Acute, early & chronic exposure**
- **384-well capacity**
- **HepG2**

Cellumen: CellCiphr™ Cytotoxicity Panel

- 10-point conc-response (200 μM-39 nM) in duplicate
- Three time points (1 hr, 24 hr, 72 hr)
- 11 endpoints per assay

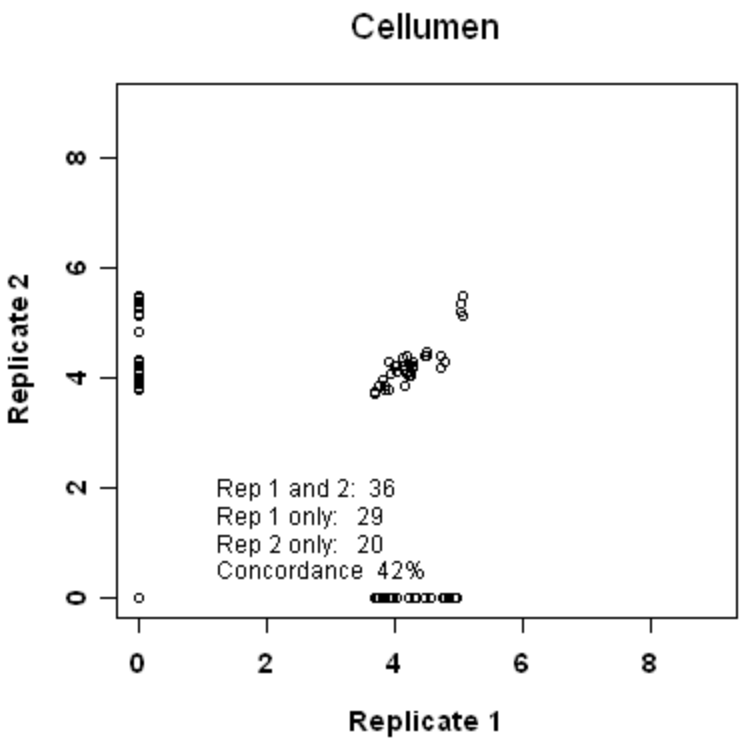
Biomarker	Measurement	Positive Control	Z'
Stress Pathway	Phospho-c-jun	Anisomycin	0.63
Oxidative Stress	Phospho-Histone H2A.X	Camptothecin	0.7
Mitochondrial Function	Mitochondrial membrane potential	CCCP	0.55
Mitochondrial Mass	Mitochondrial mass	CCCP	0.35
Cell Loss	Cell number	Camptothecin	0.56
Cell Cycle	DNA content	Paclitaxel	0.54
DNA Degradation	DNA structure	Paclitaxel	0.6
Nuclear Size	Area of nuclear region	Paclitaxel	0.63
DNA Damage	Detection of p53	Camptothecin	0.43
Mitotic Arrest	Phospho-Histone-H3	Paclitaxel	0.63
Cytoskeletal Integrity	Detection of α-tubulin	Paclitaxel	0.3

Cellumen: What is Being Measured?

- Cellular toxicity phenotypes in a transformed cell line
- False positives
  - Imaging artifacts
  - Fluorescent compounds
  - Statistical vs biological significance
  - Operational
- False negatives
  - Solubility
  - Cytotoxicity
  - Lack of biotransformation
  - Operational

Cellumen: Data Calculation

- Data not normalized to controls (with a few exceptions)
- Fit to 3- or 4-parameter Hill equation
- AC50 reported with these rules:
  - For Cell Loss, AC50 is reported as calculated
  - For other endpoints, if AC50 for endpoint is > AC50 for Cell Loss at the corresponding exposure time, AC50 for endpoint is set to 100 μM (to account for imaging artifacts of cytotoxicity
- Issues:
  - Lack of positive controls for all endpoint/time combinations
  - Large differences in maximal response
  - noisy curves due in part to effects of cytotoxicity



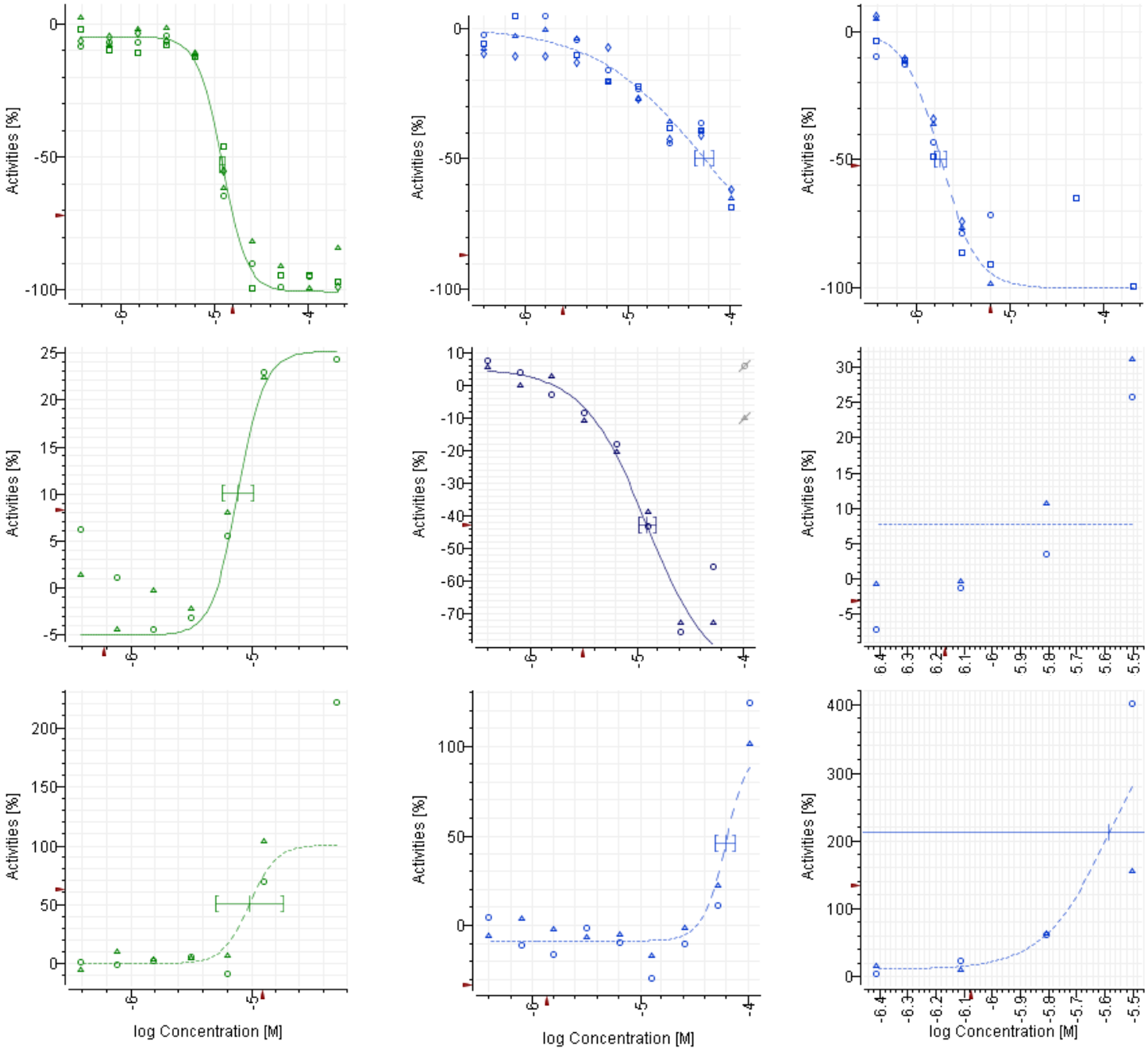
Cellumen: Data Examples

United States Environmental Protection Agency

Cell Loss

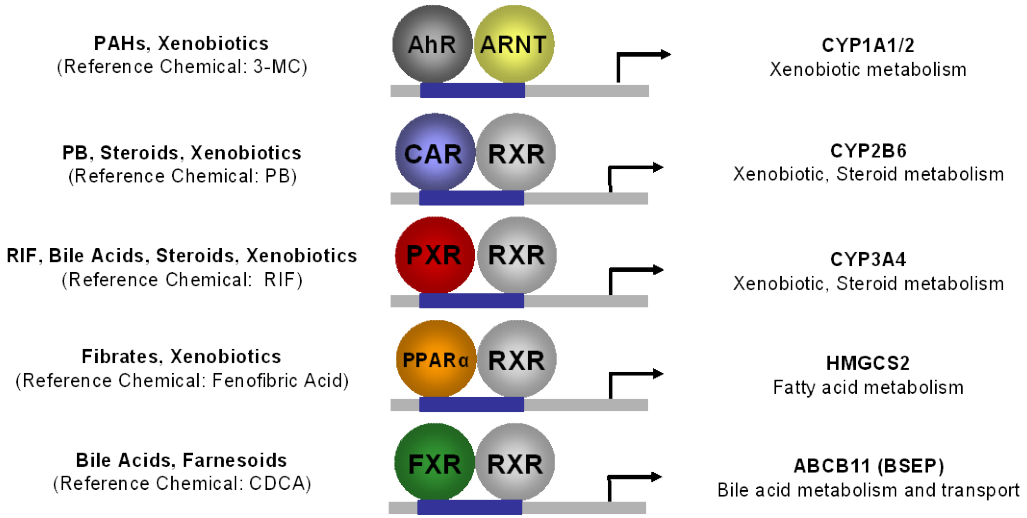
Mitochondrial Membrane Potential

DNA Damage



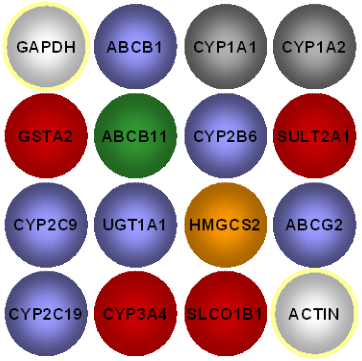
CellzDirect: XME Gene Expression in Primary Human Hepatocytes (Abstract 22)

- Primary human hepatocytes from two donors used
- Cells exposed for 6, 24, and 48 hr; medium/chemical refreshed daily
- Concentrations tested: 40, 4, 0.4, 0.04, and 0.004 µM
- 16 Genes measured in multiplexed RNAse protection assay (qNPA)
- Genes targeted XME and transporters



Target Gene Categories

CYP450 (6)
Transporter (4)
Phase II Metabolism (3)
Cholesterol Synthesis (1)
Endogenous control (2)

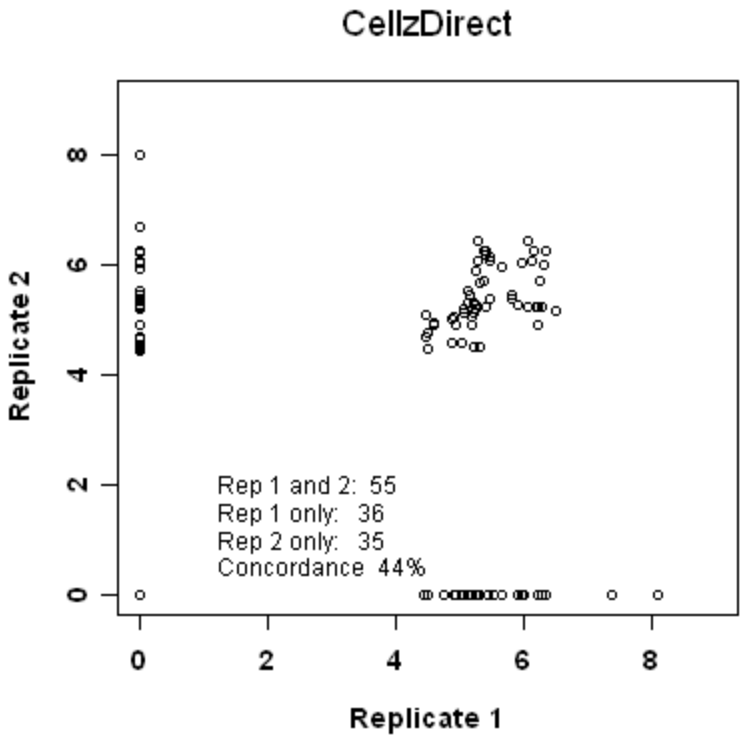


CellzDirect: What is Being Measured?

- Up/down regulation of mRNA for XME and transporters in primary human hepatocytes
- False positives:
  - General effect of cytotoxicity on transcriptional activity
  - Statistical vs biological significance
  - Operational
- False negatives:
  - Solubility
  - Cytotoxicity
  - Operational
  - Lack of biotransformation
  - Inter-individual donor variation

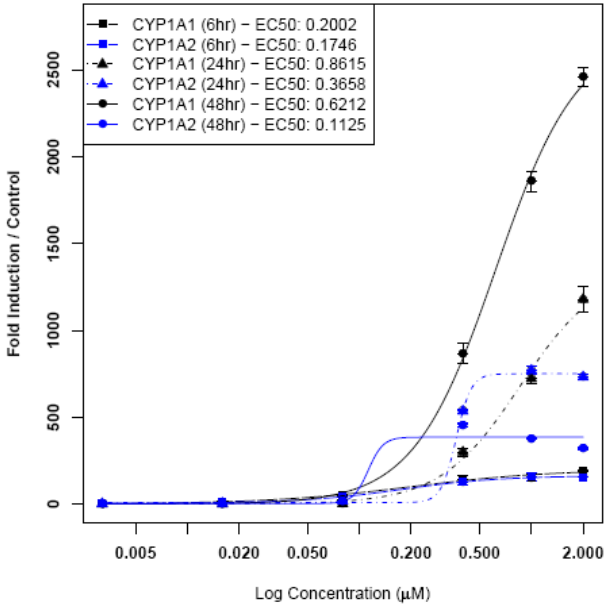
CellzDirect: Data Calculation

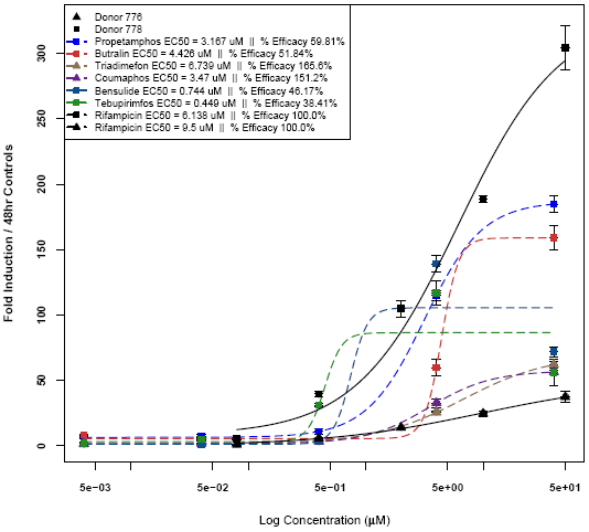
- Normalized to solvent control; expressed as fold-change
- Curves fit to Hill equation
  - Upper and lower limits defined by minimum and maximum responses observed over dataset of a particular gene/donor/time
- LELs determined
- Large variations in maximal responses
  - Biological vs statistical significance
- Measuring endogenous promoter activity reflects complex, multifactorial regulation of gene expression
- 6 hr exposure data not provided due to high variability associated with limited time for gene induction



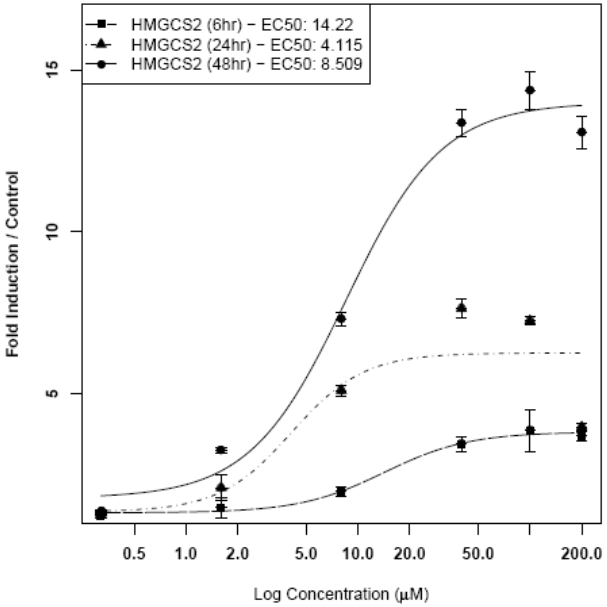
CellzDirect: Data Examples

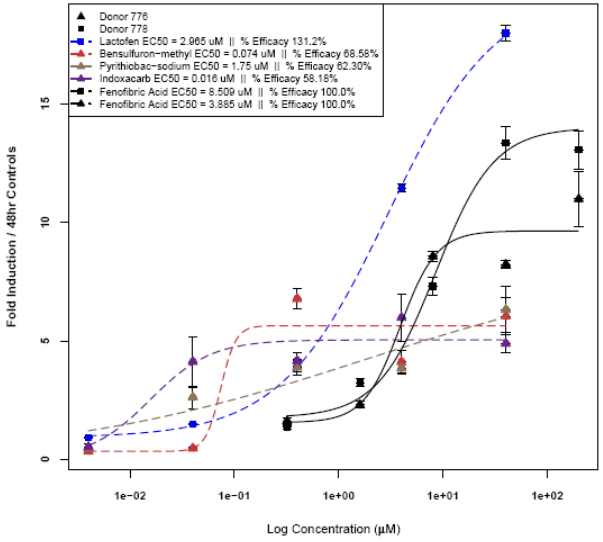
CYP1A1-AhR



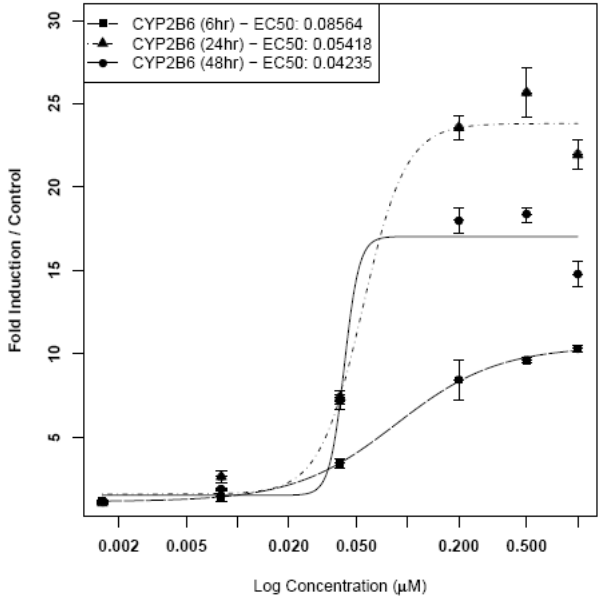


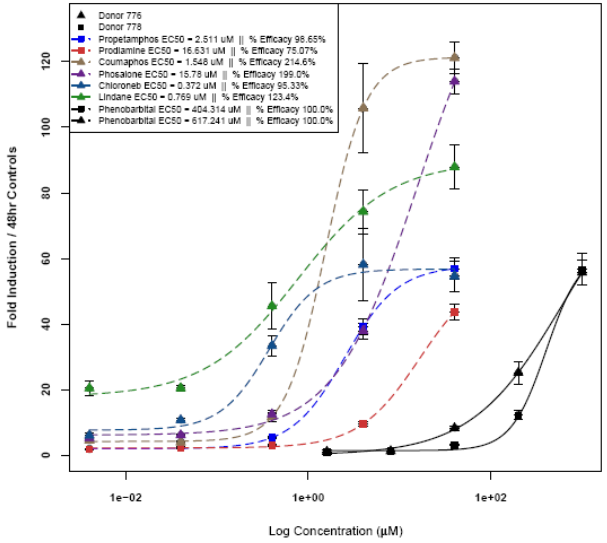
HMGCS2-PPARα





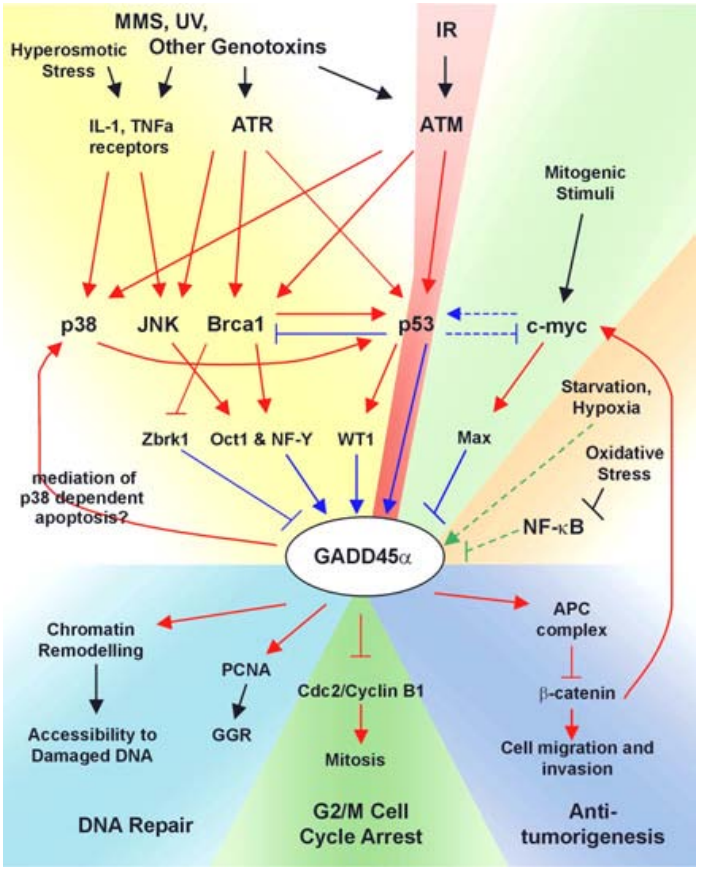
CYP2B6-CAR

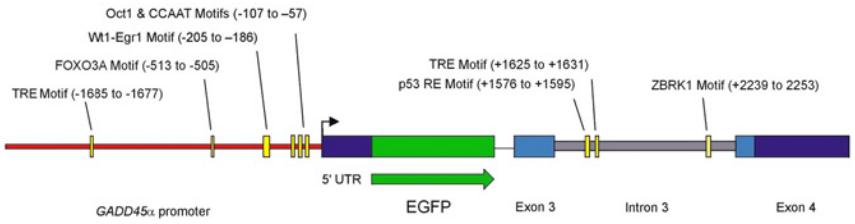




Gentronix: GADD45a Reporter Gene Assay for DNA Damage (Abstract 41)

- TK6 cell line expressing GFP under control of GADD45a promoter
- Cells exposed at 200, 100, 50 μM for 24 and 48 hr
- Cytotox assay to discount artifacts
- Retested at lower conc if cytotoxic





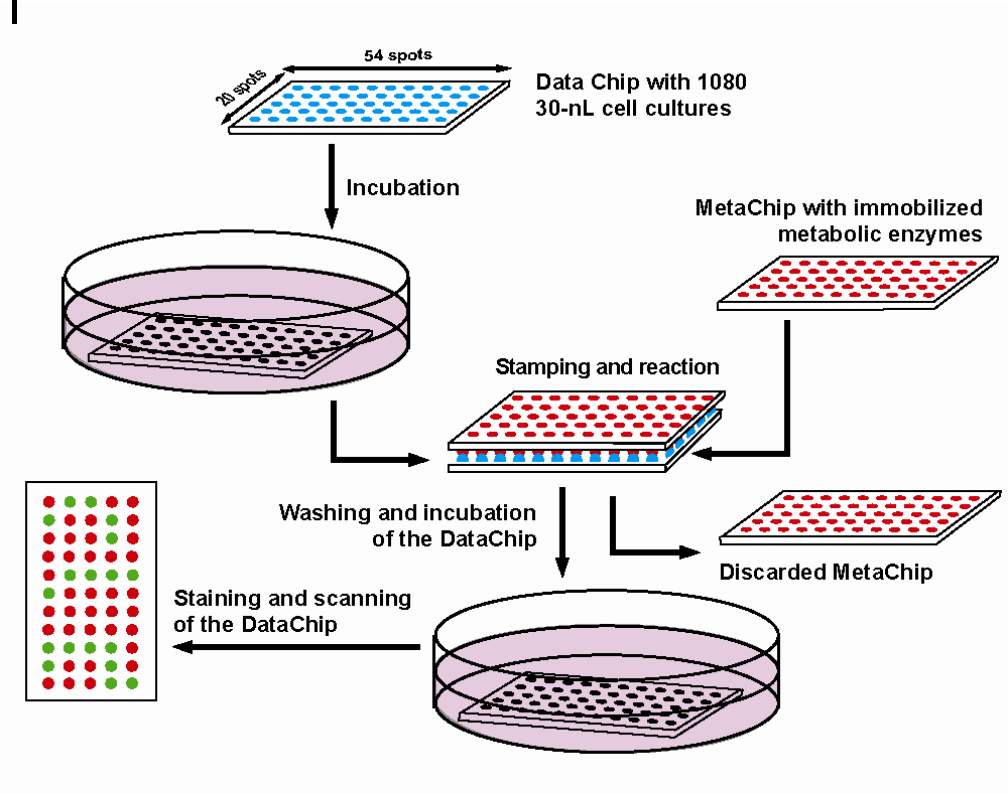
Gentronix: Data Calculations

- If induction of GFP fluorescence >50%1 test called genotoxic
- If over 2 or 3 concentrations, strongly genotoxic
- LELs calculated
- Replicate analysis: no actives among replicates

- GADD45a promoter activity in transformed cell line
- False positives:
  - Cytotoxicity resulting in general transcriptional activity
  - Cellular stress other than DNA damage
  - Statistical vs biological validation
  - Operational
- False negatives:
  - Solubility
  - Cytotoxicity
  - Lack of biotransformation
  - Operational

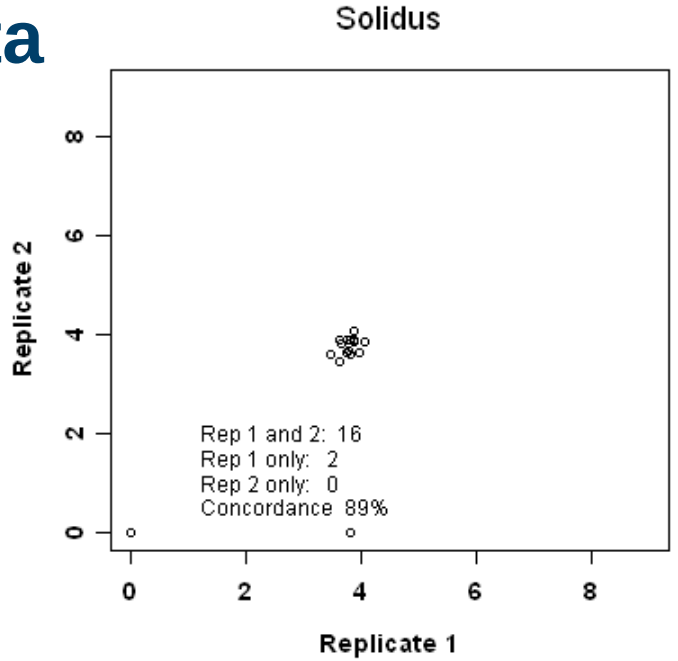
Solidus: Biotransformation Chip and Effect of Cytotoxicity (Abstract 30)

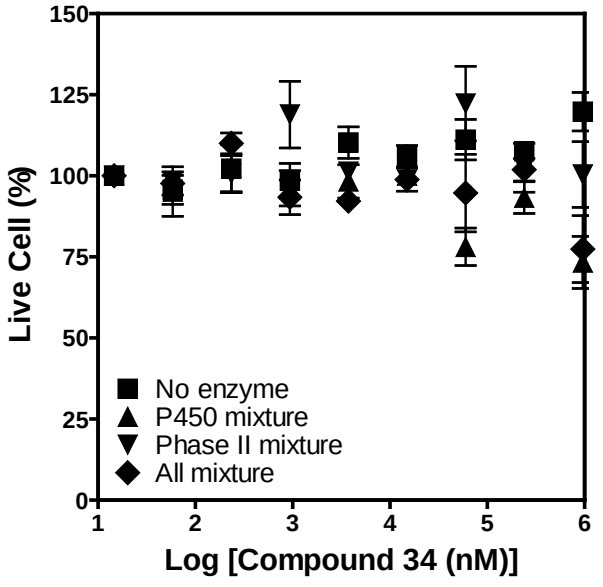
- Alginate-immobilized Phase I and Phase II enzymes
- ToxCast_320 exposed 6 hr to:
  - Control
  - Ph I
  - Ph II
  - Ph I and Ph II
- 960 uM high conc/4-fold serial dilutions/9 concentrations/5 replicates
- Cytotoxicity in Hep3B measured 48 hr later

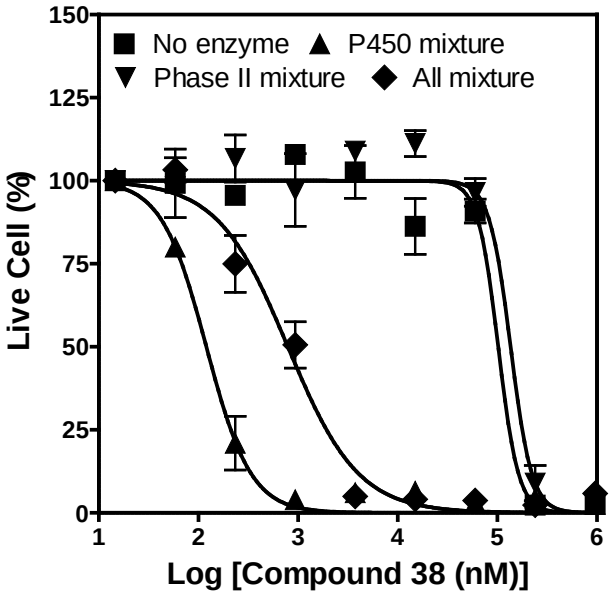


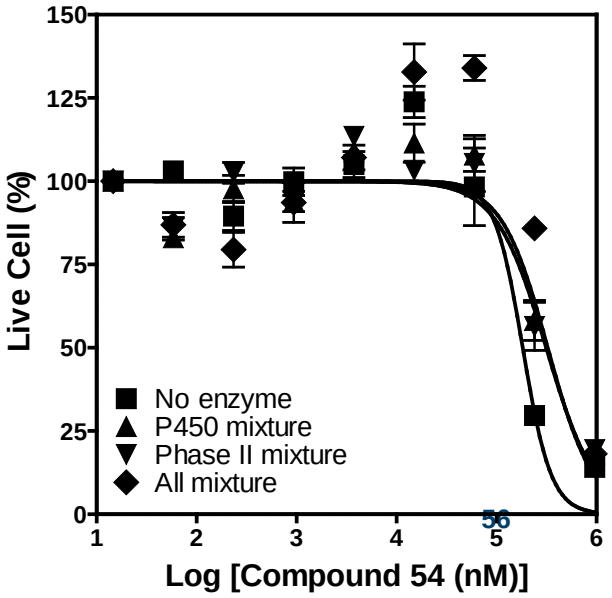
Solidus: Example Data

- Data normalized to control values
- Concentration-response data fit to Hill equation
- LC50 determined for each assay condition









Solidus: What is Being Measured?

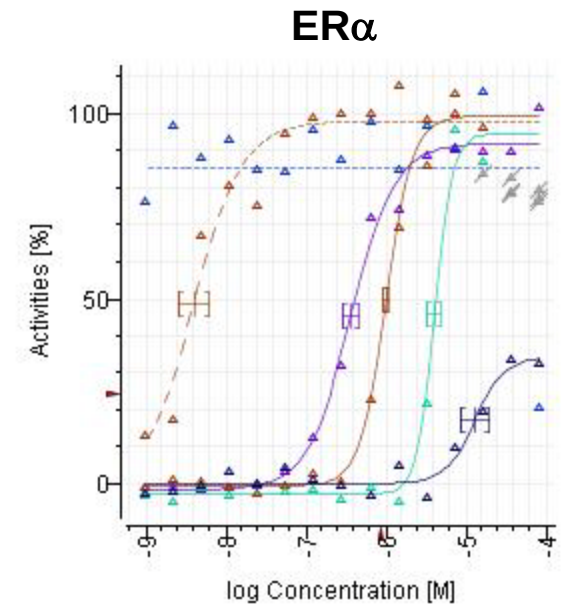
- Effect of Phase I and Phase II enzymes on cytotoxicity activity of chemicals against a transformed cell line
- False positives:
  - Possibly not optimized Phase I and/or Phase II mix
  - Operational
- False negatives
  - Solubility
  - Possibly not optimized Phase I and/or Phase II mix
  - Availability of compound from alginate-immobilized enzyme matrix
  - Operational

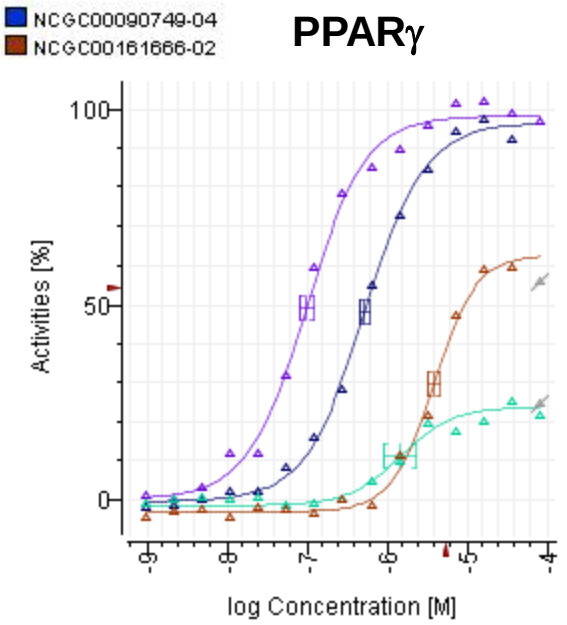
NCGC Reporter Gene Assays

- Nuclear Receptors
  - GAL4 System (ligand detection assay)
  - 11 human receptors
  - 1 rat (PXR)
  - β-lactamase reporter gene assays except:
  - PXR assays are luciferase reporter gene assays
- p53 Reporter Gene assay
  - β-lactamase reporter gene assay
- Parental cell lines mostly HEK293 (also HeLa and DPX-2)
- 12-15 point concentration-response curves (single replicate)

NCGC: Data Calculations

- Data normalized to reference compound effect
- Curves fit to 3- or 4-parameter Hill equation
- Artifacts removed where obvious fluorescence or cytotoxity detected
- Required at least 25% efficacy of control compound to calculate AC50
- AC50 values provided
- Antagonist format assays challenging due to effects of cytotoxicity
- LXR assay problematic—contaminated with GR reporter line?





NCGC Assays: What is Being Measured?

- NR assays are ligand-detection assays
- False positives
  - Fluorescent compounds
  - Statistical vs biological significance
  - Gal4_NR-LBD not physiological
  - Cytotoxicity (antagonist format)
  - Operational

- False negatives
  - Fluorescent compounds
  - Cytotoxity
  - Gal4_NR-LBD not physiological
  - Operational
  - Lack of biotransformation

Additional Data Sets To Be Added Soon:

- NHEERL
  - Zebrafish developmental toxicity (Padilla) Poster
  - Neurite outgrowth and neuronal proliferation (Mundy and Shafer)
  - ES cell differentiation (Hunter)
- Plasma protein binding and hepatocyte clearance (Thomas)
- PPARα and AhR (NCGC)
- Primary rat hepatocyte HCS Cell Health (Cellumen)